# Restyling Alternaria 

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General introduction

## GENERAL INTRODUCTION

The fungal genus Alternaria is an omnipresent dematiaceous hyphomycete which forms darkcoloured, multicellular conidia (phaeodictyospores). It includes saprophytic, endophytic and pathogenic species, and is associated with a wide variety of substrates including seeds, plants, agricultural products, humans, soil and the atmosphere. The pathogenic species include multiple serious plant pathogens, causing major losses on a wide range of crops (Thomma 2003), while others are again known as important post-harvest pathogens (Serdani et al. 2002, Kang et al. 2002), or causative agents of phaeohyphomycosis in immuno-compromised patients (Pastor \& Guarro 2008). When occurring indoors, they are also common allergens in humans causing hypersensitivity reactions, which can eventually lead to asthma (Downs et al. 2001).

Molecular studies reveal that the Alternaria complex currently comprises the genera Alternaria, Chalastospora, Crivellia, Embellisia, Nimbya, Stemphylium, Ulocladium, Undifilum and the recently described genus Sinomyces (Fig. 1). Several genera within this complex are non-monophyletic and Alternaria species cluster in multiple distinct speciesclades, which are not always correlated with species-groups based upon morphological characteristics. Alternaria alternata, the most commonly reported species in literature and type species of the genus Alternaria, also comprises one such a species-group (Simmons 1995). These small-spored Alternaria species can, however, not be distinguished based on molecular techniques alone (Peever et al. 2004, 2005, Andrew et al. 2009). As A. alternata is considered as one of the most prolific producers of fungal allergens (Horner et al. 1995, Pulimood et al. 2007, Kuna et al. 2011), and this species complex contains multiple hostspecific pathogenic strains (Kohmoto \& Otani 1991), a correct identification is of utmost importance.

## Taxonomic history

The genus Alternaria was first described with A. tenuis as the type, and only species (Nees 1816). The characteristics of the genus included the production of dark-coloured dictyospores in chains, and a beak of tapering apical cells. The genus was initially not recognized and $A$. tenuis was erroneous synonymised with Torula alternata (Fries 1832), and multiple new phaeodictyosporic hyphomycetous genera were described, such as Macrosporium (Fries 1832), Stemphylium (Wallroth 1833) and Ulocladium (Preuss 1851), further complicating the taxonomic resolution in this group of fungi. Several re-descriptions of these genera (Saccardo 1886, Elliot 1917) resulted in a growing number of new species. Due to ambiguities in the description of $A$. tenuis (Nees 1816), both $A$. tenuis and $T$. alternata were later synonymised under Alternaria alternata (Keissler 1912), and Macrosporium was synonymised under Alternaria after re-examination of their respective type species (Wiltshire 1933). Furthermore, the genus concept of Stemphylium was re-defined (Wiltshire 1938) and a later review of the genus Alternaria and related species (Joly 1964) placed a lot of atypical strains back into Alternaria.

Fig. 1. Bayesian 50 \% majority rule consensus tree based on GAPDH sequence data from Pryor \& Bigelow (2003), Hong et al. (2005), Andersen et al. (2009), Pryor et al. (2009), Runa et al. (2009), Lawrence et al. (2011), and Wang et al. (2011). The Bayesian posterior probabilities (PP) $>0.75$ are presented at the nodes; thickened lines indicate a PP of 1.0. The blue boxes indicate the Alternaria species-groups. The tree was rooted with Setosphaeria pedicellata.
GAPDH locus
114 isolates, 474 characters
GTR + y distribution model
207 unique site patterns

Alternaria I

Ulocladium

Alternaria II

Embellisia III

Embellisia IV
0.94 AY278821 S. vesicarium

L AY278823 P. herbarum
Stemphylium
0.1

The current concepts of the genera started with the seminal paper "Typification of Alternaria, Stemphylium and Ulocladium" (Simmons 1967) followed by multiple Alternaria essays, where two additional asexual genera were described as close relatives of Alternaria, namely Embellisia (Simmons 1971) and Nimbya (Simmons 1989). A life-time work on Alternaria taxonomy eventually resulted in The Alternaria Identification Manual (Simmons 2007), with morphological descriptions of 275 recognised Alternaria species. Here again three new genera, Alternariaster, Chalastospora, and Teretispora, were segregated from Alternaria, based on morphological characters. Meanwhile, the sexual genus Crivellia with Brachycladium as asexual morph was described (Inderbitzin et al. 2006), to accommodate a former Pleospora species. Based on morphological and molecular data Crivellia was shown to belong to the Alternaria complex rather than to Pleospora. The genus Undifilum was described to accommodate two species formerly classified in Embellisia and Helminthosporium (Pryor et al. 2009). Based on molecular data Undifilum formed a distinct clade from the other Embellisia species and species in Alternaria, Ulocladium, Nimbya and Crivellia. Morphologically Undifilum resembled Embellisia, with the exception of its typical germ tube formation. The latest genus in the Alternaria complex, Sinomyces, was described to accommodate a former Ulocladium species and two new species from China (Wang et al. 2011). The generated molecular data supported them as a distinct clade within the Alternaria complex. Morphologically Sinomyces resembled Ulocladium, but Sinomyces showed no, or only 1 to 2 geniculate, sympodial proliferations.

## Economic importance

## Plant pathogens

The genus Alternaria contains multiple species which are known as serious plant pathogens on a variety of crops (Thomma 2003) and ornamentals (Chase 2005). The aerial parts of the plant are mostly attacked, and infection will often start with a small circular dark leaf spot which can reach 1 cm or more in diameter as the disease progresses. Since the growth-rate of Alternaria is related to changing environmental conditions, a typical growth pattern of concentric rings is observed in the leaf spot. Alternaria infections on roots, tubers, stems and fruits often show dark, sunken lesions (Laemmlen 2001). A yellow halo around the leaf spot can also be present, caused by diffusing toxins produced by the different Alternaria species. Most plant pathogenic Alternaria species are known to produce secondary metabolites, mostly phytotoxins, which can play an important role in the pathogenesis of plants (Logrieco et al. 2009). Some species, like A. alternata, produce several mycotoxins in infected plants, which can be toxic for animals and even humans (Logrieco et al. 2009). Furthermore, A. alternata can carry host-specific toxin gene clusters on a conditionally dispensable (CD) chromosome. This CD chromosome can be lost (Johnson et al. 2001) or gained (Salamiah et al. 2001, Masunaka et al. 2005, Akagi et al. 2009), making an isolate either nonpathogenic or pathogenic to the specific host affected by the toxin which it carries.

Besides $A$. alternata, multiple plant pathogens are found in the $A$. porri species-group, e.g. A. porri, A. solani, A. tomatophila. Alternaria porri causes purple blotch of onion, a very destructive disease of onions worldwide. The disease causes a significant reduction in seed and bulb yield, with seed losses of up to $100 \%$ (Abo-Elyousr et al. 2014). Alternaria solani is the causative agent of early blight of potato. This very common disease, which can be found in most potato-growing countries, can cause considerable defoliation. The disease typically reduces yields by $\sim 20 \%$, but yield reductions of up to $80 \%$ have been reported (Horsfield et al. 2010). Alternaria tomatophila is known for causing early blight of tomato, attacking the

Table 1. Important plant pathogenic Alternaria species and the names of the diseases they cause.

| Alternaria species | Disease |
| :--- | :--- |
| A. arborescens | Tomato stem canker |
| A. brassicae | Brassicaceae leaf spot |
| A. brassicicola | Brassicaceae black spot |
| A. citri | Rough lemon leaf spot |
| A. dauci | Carrot leaf blight |
| A. gaisen | Japanese pear black spot |
| A. longipes | Tobacco brown spot |
| A. mali | Apple blotch |
| A. petroselini | Parsley leaf blight |
| A. porri | Onion purple blotch |
| A. radicina | Carrot black rot |
| A. solani | Potato early blight |
| A. tomatophila | Tomato early blight |
| A. triticina | Wheat leaf blight |

leaves, stems and fruit. This airborne pathogen has spread worldwide, mainly affecting field crops. When left untreated the damage can result in plant defoliation in excess of $60 \%$ (Zitter \& Drennan 2005). An overview of important plant pathogenic Alternaria species and the names of the diseases they cause are given in Table 1.

## Post-harvest pathogens

A variety of rots and decay caused by fungi or bacteria can be found on stored products. These post-harvest diseases can start before or after harvesting. Plants or fruits infected in the field may not develop symptoms until stored, and the presence of high temperatures and high moisture during storage can stimulate the infections to continue to develop on fruits and vegetables. Penetration can occur through natural openings, but most post-harvest pathogens need wounds, cuts, or bruises caused during harvesting to infect the host (Barkai-Goland 2001). Post-harvest losses of fruit and vegetables can reach up to $25 \%$ of the total production in industrialized countries and even more than $50 \%$ in developing countries (Nunes 2012). Alternaria core rot of apples and Alternaria black rot of citrus are two examples of serious post-harvest diseases caused by isolates of the $A$. arborescens, $A$. infectoria and $A$. tenuissima species-groups (Serdani et al. 2002, Kang et al. 2002). In contrast to the host specificity observed in plant diseases, no host specificity seems to exist in the post-harvest diseases with these different small-spored Alternaria species (Kang et al. 2002).

## Allergens

Airborne spores, hyphae and fragments of fungi are small enough to be inhaled into the lower airways (Randriamanantany et al. 2010). As Alternaria is omnipresent in the environment, Alternaria species are frequently associated with hypersensitivity pneumonitis, asthma and allergic fungal rhinitis and sinusitis. Allergic rhinitis is the most common form of noninfectious rhinitis (Randriamananany et al. 2010), while allergic (extrinsic) asthma is the most common form of asthma, affecting over $50 \%$ of the 20 million asthma sufferers (Salo et al. 2006). The reported prevalence of Alternaria sensitivity varies significantly among different studies. In the European Community Respiratory Health Survey I (ECRHS), the prevalence of a positive skin test for Alternaria, tested on 18102 adults at 35 centers in 15 well-developed countries
worldwide, was 3.3 \%, but ranged from 0.2 \% to 14.4 \% (Bousquet et al. 2007). The French Six Cities study showed a $2.8 \%$ prevalence of a positive skin test for Alternaria sensitization in children (Radriamanantany et al. 2010). This variation probably arose from differences in the diagnostic methods used in the various studies (skin prick vs. test for specific IgEs), selection of patients with different ages (children $v s$. adolescents and adults) and the diseases analysed (asthma vs. rhinitis), climate where the study took place (variation in humidity and temperature) and other environmental factors. The Alternaria species considered as the main airborne allergen is Alternaria alternata (Horner et al. 1995, Pulimood et al. 2007, Kuna et al. 2011).

## Phaeohyphomycosis

Phaeohyphomycosis comprises fungal infections caused by dematiaceous (pigmented) filamentous fungi. The fungus often occurs on the skin where it can form nodules or cysts but is also capable of invading the deeper tissues and even the brain where hypha, yeast-like cells or a combination of these morphologic characteristics can be observed. The fungus is not capable of penetrating the skin itself, but needs a cut or wound to cause infection. Usually it is caused by contaminated material, such as a splinter or plant matter, which enters the skin during an injury. These opportunistic fungal infections mainly affect immuno-compromised people.

A recent review describes 210 reported cases of Alternaria infections in humans between 1933 and 2007 with the majority (almost $75 \%$ ) being cutaneous and subcutaneous infections followed by oculomycosis, invasive and non-invasive rhinosinusitis and onychomycosis (Pastor \& Guarro 2008). In the majority of the reported cases identification to species level was not performed. The most common clinical species is $A$. infectoria, although $A$. alternata and $A$. tenuissima are often wrongly mentioned as causative agent. Other Alternaria species mentioned rarely as causative agents are, for example, A. chlamydospora, A. longipes and A. dianthicola (Pastor \& Guarro 2008). A study on phaeohyphomycosis due to Alternaria species in transplant recipients (Boyce et al. 2010) also concluded that Alternaria species are rare, but increasingly recognised as causing opportunistic infections among highly immunocompromised transplant recipients.

## Species complexes

The species-group delineation within Alternaria based on morphological characteristics was first introduced by Elliott (1917), who described six species-groups based on spore similarity. Others divided the genus in two groups, one forming chains of spores with a relative short beak and one with conidia containing a long filiform beak which seldom form conidial chains (Angel 1929, Wiltshire 1933). Later on, a subdivision based on length of the conidial chains and length of the conidial beak was suggested, resulting in the sections longicatenatae, brevicatenatae and noncatenatae (Neergaard 1945). Based partly on these first subdivisions, a key to 14 speciesgroups using characters of the conidium, the pattern of chain formation, and the nature of the apical extensions of conidium cells was described (Simmons 1992) and several hundreds of small-spored chain-forming pear isolates were divided into nine groups based on sporulation patterns (Simmons \& Roberts 1993). The first descriptions of the A. alternata, A. tenuissima, A. cheiranthi and A. brassicicola species-groups were made by Simmons (1995). These key morphology and sporulation characteristics, observed under strict standardised laboratory conditions, are used in the treatment of the genus in The Alternaria Identification Manual (Simmons 2007). Section one of the Manual contains the relatively large-spored species, further

Table 2. Molecular species-groups within Alternaria and the species described herein. ${ }^{1}$

| Species-group | Species |
| :--- | :--- |
| A. alternantherae | A. alternantherae, A. celosiae, A. perpunctulata |
| A. alternata | A. alternata, A. arborescens, A. citriarbusti, A. citrimacularis, A. colombiana, |
|  | A. destruens, A. dumosa, A. gaisen, A. interrupta, A. limoniasperae, A. longipes, |
|  | A. mali, A.perangusta, A.tangelonis, A. tenuissima, A. toxicogenica, A. turkisafria |
| A. brassicicola | A. brassiscola, A. japonica, A. mimicula, Embellisia conoidea |
| A. infectoria | A. arbusti, A.conjuncta, A.ethzedia, A.infectoria, A. intercepta, A. metachromatica, |
|  | A. oregonensis, A. photistica, A. tritici-maculans, A. triticina, A. viburni |
| A. porri | A. blumae, A. capsici, A. carthami, A. crassa, A. cucumerina, A. dauci, |
|  | A. euphorbiicola, A. limicola, A. linicola, A. macrospora, A. porri, A. protenta, |
|  | A. pseudorostrata, A. sesami, A. solani, A. tagetica, A. zinniae |
| A. radicina | A. carotiincultae, A. petroselini, A. radicina, A. selini, A. smyrnii, Ulocladium |
|  | lanuginosum |
| A. sonchi | A. cinerariae, A. sonchi |

${ }^{1}$ Based on Pryor \& Gilberston (2000), Pryor \& Michailides (2001), Chou \& Wu (2002), Pryor \& Bigelow (2003), Peever et al. (2004), Hong et al. (2005), Pryor et al. (2009), Runa et al. (2009), Andersen et al. (2009), Lawrence et al. (2011).
divided by the shape of the conidium beak. Section two of the Manual contains the species with a relatively short conidium body further divided by the number of conidia produced and their chain formation.

Modern, molecular-based studies revealed that Alternaria species do cluster in several distinct species clades, which do not always correlate with species-groups delineated based on morphological characteristics. Currently seven species-groups are recognised based on molecular phylogenies (Fig. 1), which together harbour 58 Alternaria species (Table 2). The A. porri and A. sonchi species-groups reside in Section one in the Identification Manual, large-spored conidia, and Section two, the small-spored conidia, harbours the A. alternata, A. radicina, A. brassicicola and $A$. infectoria species-groups. The $A$. alternantherae species-group (Lawrence et al. 2011), harbours three species which were formerly recognised as Nimbya species.

## Morphology

Alternaria is characterised as a dematiaceous hyphomycete whose phaeodictyospores, dark coloured conidia with transverse and longitudinal septa, develop at a very restricted site in the apex of distinctive conidiophores (Fig. 2A). The genus Ulocladium can be distinguished from Alternaria by its young obovoid non-beaked conidia (Fig. 2B). The newest genus Sinomyces resembles Ulocladium but is distinct by its conidiophores which only form a single conidiogenous locus, or rarely form 1 to 2 geniculate, sympodial proliferations with a single conidiogenous locus (Fig. 2C). Embellisia is characterised by the thick, dark, rigid septa in its conidia and the paucity of longisepta (Fig. 2D). Undifilum resembles Embellisia, except that most of the isolates produce the toxic alkaloid swainsonine, and the conidia produce germ tubes that are wavy or undulating in their growth until branching (Fig. 2E). Chalastospora can be distinguished by their narrowly ellipsoid conidia, rarely with transverse eusepta and lacking longitudinal septa (Fig. 2F). Crivellia is characterised by having cylindrical phragmoconidia (Fig. 2G) and Nimbya


Fig. 2. Conidia and conidiophores: A. Alternaria tenuissima. B. Ulocladium obovoideum.
C. Sinomyces alternariae. D. Embellisia allii. E. Undifilum bornmuelleri. F. Chalastospora cetera.
G. Crivellia papaveraceae. H. Nimbya scirpicola. I. Stemphylium botryosum. Scale bars $=10 \mu \mathrm{~m}$.
has distoseptate conidia with internal compartmentation (Fig. 2H). Stemphylium can easily be identified on the basis of the percurrent proliferation of its conidiophores (Fig. 2I), while all other genera within the Alternaria complex display geniculate, sympodial proliferation. Identification of Alternaria species based solely on conidial morphology is complicated since the morphological characters are very sensitive to variation in culture conditions. Highly standardised culture methods are obligatory but still variation within the same species can be seen when cultured by two independent laboratories.

## Molecular studies

Molecular studies revealed that the Alternaria complex comprises the genera Alternaria, Chalastospora, Crivellia, Embellisia, Nimbya, Stemphylium, Ulocladium, Undifilum and the recently described genus Sinomyces (Fig. 1). In this complex several genera seem to be nonmonophyletic and numerous studies reveal that Alternaria species cluster in multiple distinct species clades, which are not always correlated with species-groups based upon morphological characteristics alone. Pryor \& Gilbertson (2000) tried to elucidate the relationship between Alternaria, Ulocladium and Stemphylium species by using parts of the nuclear internal transcribed spacer (ITS), mitochondrial small subunit (mtSSU) and nuclear small subunit (SSU; 18S rRNA gene) sequences. They revealed that Stemphylium was phylogenetically distinct from Ulocladium and Alternaria, which clustered together in one clade. Furthermore, they confirmed the species-groups by obtaining distinct species clades. Chou \& Wu (2002) could discriminate the filament-beaked Alternaria from the other members of Alternaria and de Hoog \& Horré (2002) studied medical Alternaria and Ulocladium species using ITS sequence data. As these loci do not clearly resolve the relationships among closely related species within the groups, additional analyses incorporating more variable genetic loci were suggested to develop a more robust phylogeny of the Alternaria species involved. By using sequences of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene or / and a gene for the major Alternaria allergen (Alt a 1), sometimes in addition to the ITS or mtSSU sequences, multiple investigators attempted to elucidate the phylogenetic relationship between Alternaria species-groups and their related genera (Pryor \& Bigelow 2003, Hong et al. 2005a, Inderbitzin et al. 2006, Pryor et al. 2009, Runa et al. 2009, Lawrence et al. 2011, Wang et al. 2011). The grouping including A. alternata, A. brassicicola, A. infectoria, A. porri, A. radicina, Embellisia and Ulocladium species-groups was strongly supported by these studies and even two new species-groups were described. These included the $A$. sonchi species-group (Hong et al. 2005a), consisting of $A$. sonchi and $A$. cinerariae and in the latest taxonomic study (Lawrence et al. 2011) the A. alternantherae species-group consisting of three former Nimbya species, A. alternantherae, A. perpunctulata and $A$. celosiae. Also the new genera Crivellia (Inderbitzin et al. 2006), Undifilum (Pryor et al. 2009) and Sinomyces (Wang et al. 2011) were described by employing DNA sequence data. Unfortunately, species relationships within the species-groups remained poorly resolved. A study by Peever et al. (2004) based on multiple genetic regions of the small-spored citrus-associated species of Alternaria revealed that many of the morphological species described (Simmons 1999) were non-monophyletic. Results were based on sequence variation of five genomic regions, namely the mitochondrial LSU, the beta-tubulin and endopolygalacturonase gene (endoPG) and the anonymous gene regions OPA1-3 and OPA2-1 (Peever et al. 2004). Sequence analyses of five other genes, actin, calmodulin, chitin synthase, translation elongation factor 1-alpha (TEF1) and trihydroxynaphthalene reductase, yielded no significant variation among the small-
spored isolates. The authors advocated reducing all small-spored citrus-associated isolates into a single phylogenetic species: A. alternata. Follow-up studies (Peever et al. 2005, Andrew et al. 2009) using sequence data from endoPG, OPA1-3 and OPA2-1 or OPA10-2 yielded identical results. No associations were found between host or geographic associations and phylogenetic lineages, and no strict congruence between morphology and phylogenetic lineage was found within the $A$. alternata species-group. Furthermore, different molecularbased identification methods such as RAPD (Roberts et al. 2000), PCR-RFLP (Pryor \& Michailides 2002), AFLP (Dini-Andreote et al. 2009), ISSR (Hong et al. 2006) and restriction mapping of the IGS region (Hong et al. 2005b), used in an attempt to discriminate within the species-groups, demonstrated no results with improved resolution. However, a RAPD fragment pattern analysis of 260 small-spored Alternaria species and species-groups (Roberts et al. 2000), could distinguish between A. gaisen, A. longipes, the A. tenuissima group, the A. arborescens group and the $A$. infectoria group. Recently, Roberts et al. (2011) examined the differential gene expression in $A$. gaisen exposed to a dark and light regime. The authors discovered that partial sequence analysis of the gene L152, with similarity to aegerolysin, could resolve A. alternata, A. gaisen, A. yaliinficiens, A. arborescens, A. tenuissima and $A$. brassicicola. It was concluded that additional analyses on a broader set of isolates including related genera and species should be performed to determine if this gene would be a potential key for discriminating among all Alternaria species.

## OUTLINE OF THESIS

Previous studies on Alternaria focused mainly on specific species-groups, species associated with specific hosts / substrates, or used a very limited sampling of representative species. In 2007, Emory G. Simmons published The Alternaria Identification Manual and subsequently kindly donated his complete collection of Alternaria and alternaria-like cultures, many representing ex-type or reference strains, to the CBS culture collection. This morphologically well-characterised collection formed a solid foundation for the molecular taxonomic revision of the genus Alternaria and related genera presented in this thesis. With the phylogenies and classifications presented herein, more robust and understandable taxonomy and nomenclature in Alternaria and allied genera are created, which will serve as a starting point for applied research conducted by plant pathologists, breeders and medical mycologists in the field.

Chapter 1 provides an introduction to the genus Alternaria, with an overview of the history of the genus and its economic importance, and a further focus on the species complexes, their morphology, and molecular studies.

Chapter 2 focuses on the relationship of Alternaria and its closely related genera. The phylogenetic lineages within Alternaria and allied genera are delineated based on nucleotide sequence data of parts of the SSU, LSU, ITS, GAPDH, RNA polymerase second largest subunit (RPB2) and TEF1 gene regions. The genus Alternaria is divided into six monotypic lineages and 24 internal clades, which are named as sections. Thirteen genera are placed into synonymy with Alternaria and the phylogenetic relation of several other genera to Alternaria is clarified.

Chapter 3 describes the reappraisal of the genus Alternariaster, based on phylogenetic, morphological and pathological studies. The genus Alternariaster was established by Simmons
(2007) to accommodate Alternaria helianthi, the causal agent of leaf spot on Helianthus annuus (sunflower). A new species of Alternariaster was found associated with leaf spot on Bidens sulphurea (yellow cosmos) in Brazil and is formally named in this chapter.

Chapter 4 treats the Alternaria species that represent the largest section of Alternaria, section Porri. This section contains almost all Alternaria species with medium to large conidia with long beaks, some of which are important plant pathogens. A multi-gene phylogeny on parts of the ITS, GAPDH, RPB2, TEF1 and Alt a 1 gene regions, supplemented with morphological and cultural studies, forms the basis for species recognition in this section.

Chapter 5 treats the small-spored Alternaria species which reside in section Alternaria. A lot of confusion surrounds the naming of species within this section, since the naming is mostly based on morphology and host-specificity, although the molecular variation is minimal. Whole genome sequencing, combined with transcriptome profiling and multi-gene sequencing of nine gene regions, SSU, LSU, ITS, GAPDH, RPB2, TEF1, Alt a 1, endoPG and OPA10-2, is used to create a clear and stable species classification in this section. A sequence-based identification guide is provided for the species that we recognise in section Alternaria.

Chapter 6 investigates the molecular diversity of indoor Alternaria isolates in the USA, and tests for recombination in these isolates, using a phylogeographic / population genetic approach. Isolates collected throughout the USA were identified using ITS, GAPDH and endoPG gene sequencing, followed by genotyping and population genetic inference of the section Alternaria isolates together with 37 reference isolates, using five microsatellite markers.

Chapter 7 discusses the data presented in this thesis. The implications of the performed studies are placed in a broader context, with a focus on the relation between morphology and the new species classification based on molecular tools and the use of genome data contrasted against gene data.

## Alternaria redefined

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#### Abstract

Alternaria is a ubiquitous fungal genus that includes saprophytic, endophytic and pathogenic species associated with a wide variety of substrates. In recent years, DNAbased studies revealed multiple non-monophyletic genera within the Alternaria complex, and Alternaria species clades that do not always correlate to species-groups based on morphological characteristics. The Alternaria complex currently comprises nine genera and eight Alternaria sections. The aim of this study was to delineate phylogenetic lineages within Alternaria and allied genera based on nucleotide sequence data of parts of the $18 \mathrm{~S} \mathrm{nrDNA}, 28 \mathrm{~S} \mathrm{nrDNA}$, ITS, GAPDH, RPB2 and TEF1-alpha gene regions. Our data reveal a Pleospora / Stemphylium clade sister to Embellisia annulata, and a well-supported Alternaria clade. The Alternaria clade contains 24 internal clades and six monotypic lineages, the assemblage of which we recognise as Alternaria. This puts the genera Allewia, Brachycladium, Chalastospora, Chmelia, Crivellia, Embellisia, Lewia, Nimbya, Sinomyces, Teretispora, Ulocladium, Undifilum and Ybotromyces in synonymy with Alternaria. In this study, we treat the 24 internal clades in the Alternaria complex as sections, which is a continuation of a recent proposal for the taxonomic treatment of lineages in Alternaria. Embellisia annulata is synonymised with Dendryphiella salina, and together with Dendryphiella arenariae, are placed in the new genus Paradendryphiella. The sexual genera Clathrospora and Comoclathris, which were previously associated with Alternaria, cluster within the Pleosporaceae, outside Alternaria s. str., whereas Alternariaster, a genus formerly seen as part of Alternaria, clusters within the Leptosphaeriaceae. Paradendryphiella is newly described, the generic circumscription of Alternaria is emended, and 32 new combinations and 10 new names are proposed. A further 10 names are resurrected, while descriptions are provided for 16 new Alternaria sections.


Taxonomic novelties: New combinations - Alternaria abundans (E.G. Simmons) Woudenb. \& Crous, Alternaria alternariae (Cooke) Woudenb. \& Crous, Alternaria atra (Preuss) Woudenb. \& Crous, Alternaria bornmuelleri (Magnus) Woudenb. \& Crous, Alternaria botrytis (Preuss) Woudenb. \& Crous, Alternaria caespitosa (de Hoog \& C. Rubio) Woudenb. \& Crous, Alternaria cantlous (Yong Wang bis \& X.G. Zhang) Woudenb. \& Crous, Alternaria caricis (E.G. Simmons) Woudenb. \& Crous, Alternaria cinerea (Baucom \& Creamer) Woudenb. \& Crous, Alternaria didymospora (Munt.-Cvetk.) Woudenb. \& Crous, Alternaria fulva (Baucom \& Creamer) Woudenb. \& Crous, Alternaria hyacinthi (de Hoog \& P.J. Mull. bis) Woudenb. \& Crous, Alternaria indefessa (E.G. Simmons) Woudenberg \& Crous, Alternaria leptinellae (E.G. Simmons \& C.F. Hill) Woudenb. \& Crous, Alternaria lolii (E.G. Simmons \& C.F. Hill) Woudenb. \& Crous, Alternaria multiformis (E.G. Simmons) Woudenb. \& Crous, Alternaria obclavata (Crous \& U. Braun) Woudenb. \& Crous, Alternaria obovoidea (E.G. Simmons) Woudenb. \& Crous, Alternaria oudemansii (E.G. Simmons) Woudenb. \& Crous, Alternaria oxytropis (Q. Wang, Nagao \& Kakish.) Woudenb. \& Crous, Alternaria penicillata (Corda) Woudenb. \& Crous, Alternaria planifunda (E.G. Simmons) Woudenb. \& Crous, Alternaria proteae (E.G. Simmons) Woudenb. \& Crous, Alternaria scirpinfestans (E.G. Simmons \& D.A. Johnson) Woudenb. \& Crous, Alternaria scirpivora (E.G. Simmons \& D.A. Johnson) Woudenb. \& Crous, Alternaria septospora (Preuss) Woudenb. \& Crous, Alternaria slovaca (Svob.-Pol., L. Chmel \& Bojan.) Woudenb. \& Crous, Alternaria subcucurbitae (Yong Wang bis \& X.G. Zhang) Woudenb. \& Crous, Alternaria tellustris (E.G. Simmons) Woudenb. \& Crous, Alternaria tumida (E.G. Simmons) Woudenb. \& Crous, Paradendryphiella salina (G.K. Sutherl.) Woudenb. \& Crous, Paradendryphiella arenariae (Nicot) Woudenb. \& Crous. New names - Alternaria aspera Woudenb. \& Crous, Alternaria botryospora Woudenb. \& Crous, Alternaria brassicae-pekinensis Woudenb. \& Crous, Alternaria breviramosa Woudenb. \&

Crous, Alternaria chlamydosporigena Woudenb. \& Crous, Alternaria concatenata Woudenb. \& Crous, Alternaria embellisia Woudenb. \& Crous, Alternaria heterospora Woudenb. \& Crous, Alternaria papavericola Woudenb. \& Crous, Alternaria terricola Woudenb. \& Crous. Resurrected names-Alternaria cetera E.G. Simmons, Alternaria chartarum Preuss, Alternaria consortialis (Thüm.) J.W. Groves \& S. Hughes, Alternaria cucurbitae Letendre \& Roum., Alternaria dennisii M.B.Ellis, Alternaria eureka E.G. Simmons, Alternaria gomphrenae Togashi, Alternaria malorum (Ruehle) U. Braun, Crous \& Dugan, Alternaria phragmospora Emden, Alternaria scirpicola (Fuckel) Sivan. New sections, all in Alternaria - sect. Chalastospora Woudenb. \& Crous, sect. Cheiranthus Woudenb. \& Crous, sect. Crivellia Woudenb. \& Crous, sect. Dianthicola Woudenb. \& Crous, sect. Embellisia Woudenb. \& Crous, sect. Embellisioides Woudenb. \& Crous, sect. Eureka Woudenb. \& Crous, sect. Infectoriae Woudenb. \& Crous, sect. Japonicae Woudenb. \& Crous, sect. Nimbya Woudenb. \& Crous, sect. Phragmosporae Woudenb. \& Crous, sect. Pseudoulocladium Woudenb. \& Crous, sect. Teretispora Woudenb. \& Crous, sect. Ulocladioides Woudenb. \& Crous, sect. Ulocladium Woudenb. \& Crous, sect. Undifilum Woudenb. \& Crous. New genus - Paradendryphiella Woudenb. \& Crous.

## INTRODUCTION

Alternaria is a ubiquitous fungal genus that includes saprophytic, endophytic and pathogenic species. It is associated with a wide variety of substrates including seeds, plants, agricultural products, animals, soil and the atmosphere. Species of Alternaria are known as serious plant pathogens, causing major losses on a wide range of crops. Several taxa are also important postharvest pathogens, causative agents of phaeohyphomycosis in immuno-compromised patients or airborne allergens. Because of the significant negative health effects of Alternaria on humans and their surroundings, a correct and rapid identification of Alternaria species would be of great value to researchers, medical mycologists and the public alike.

Alternaria was originally described by Nees (1816), based on A. tenuis as the only species. Characteristics of the genus included the production of dark-coloured phaeodictyospores in chains, and a beak of tapering apical cells. Von Keissler (1912) synonymised both A. tenuis and Torula alternata (Fries 1832) with Alternaria alternata, due to ambiguities in Nees's description of A. tenuis. Two additional genera, Stemphylium (Wallroth 1833) and Ulocladium (Preuss 1851) were subsequently described for phaeodictyosporic hyphomycetes, further complicating the taxonomic resolution in this group of fungi. Several re-descriptions and revised criteria of these genera (Saccardo 1886, Elliot 1917, Wiltshire 1933, 1938, Joly 1964) resulted in a growing number of new species. Results of a lifetime study on Alternaria taxonomy based upon morphological characteristics were summarised in Simmons (2007), in which 275 Alternaria species were recognised. One species was transferred to the genus Prathoda and three new genera, Alternariaster, Chalastospora and Teretispora, were segregated from Alternaria.

Molecular studies revealed multiple non-monophyletic genera within the Alternaria complex and Alternaria species clades, which do not always correlate to species-groups based upon morphological characteristics (Pryor \& Gilbertson 2000, Chou \& Wu 2002, de Hoog \& Horré 2002, Pryor \& Bigelow 2003, Hong et al. 2005, Inderbitzin et al. 2006, Pryor et al. 2009, Runa et al. 2009, Wang et al. 2011, Lawrence et al. 2012). The A. alternata, A. brassicicola, A. infectoria, $A$. porri and $A$. radicina species-groups were strongly supported by these studies and two new species-groups, $A$. sonchi (Hong et al. 2005) and A. alternantherae (Lawrence et al. 2012) and three new genera, Crivellia (Inderbitzin et al. 2006), Undifilum (Pryor et al. 2009)
and Sinomyces (Wang et al. 2011), were described. The latest molecular revision of Alternaria (Lawrence et al. 2013) introduced two new species-groups, A. panax and A. gypsophilae, and elevated eight species-groups to sections within Alternaria. The sexual phylogenetic Alternaria lineage, the $A$. infectoria species-group, did not get the status of section, in contrast to the eight asexual phylogenetic lineages in Alternaria. The Alternaria complex currently comprises the genera Alternaria, Chalastospora (Simmons 2007), Crivellia, Embellisia, Nimbya, Stemphylium, Ulocladium, Undifilum and the recently described Sinomyces together with eight sections of Alternaria and the $A$. infectoria species-group.

The aim of the present study was to delineate the phylogenetic lineages within Alternaria and allied genera, and to create a robust taxonomy. Phylogenetic inferences were conducted on sequence data of parts of the 18 S nrDNA (SSU), 28 S nrDNA (LSU), the internal transcribed spacer regions 1 and 2 and intervening 5.8S nrDNA (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), RNA polymerase second largest subunit (RPB2) and translation elongation factor 1-alpha (TEF1) gene regions of ex-type and reference strains of Alternaria species and all available allied genera.

## MATERIALS AND METHODS

## Isolates

Based on the ITS sequences of all ex-type or representative strains from the Alternaria identification manual present at the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands (data not shown), 66 Alternaria strains were included in this study together with 61 ex-type or representative strains of 16 related genera (Table 1). Alternaria is represented by the ex-type or representative strains of the seven species-groups and species that clustered outside known Alternaria clades. Because of the size and complexity of the A. alternata, A. infectoria and $A$. porri species-groups, we only included known species; the complete speciesgroups will be treated in future studies.

Freeze-dried strains were revived in 2 mL malt / peptone ( $50 \% / 50 \%$ ) and subsequently transferred to oatmeal agar (OA) (Crous et al. 2009c). Strains of the CBS collection stored in liquid nitrogen were transferred to OA directly from $-185^{\circ} \mathrm{C}$. DNA extraction was performed using the UltraClean Microbial DNA Isolation Kit (MoBio laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions.

## Taxonomy

Morphological descriptions were made for isolates grown on synthetic nutrient-poor agar plates (SNA, Nirenberg 1976) with a small piece of autoclaved filter paper placed onto the agar surface. Cultures were incubated at moderate temperatures ( $\sim 22^{\circ} \mathrm{C}$ ) under CoolWhite fluorescent light with an 8 h photoperiod for 7 d . The sellotape technique was used for making slide preparations (Crous et al. 2009c) with Shear's medium as mounting fluid. Photographs of characteristic structures were made with a Nikon Eclipse 80i microscope using differential interference contrast (DIC) illumination. Growth rates were measured after 5 and 7 d . Colony characters were noted after 7 d, colony colours were rated according to Rayner (1970). Nomenclatural data were deposited in MycoBank (Crous et al. 2004).

## PCR and sequencing

The SSU region was amplified with the primers NS1 and NS4 (White et al. 1990), the LSU region with LSU1Fd (Crous et al. 2009b) and LR5 (Vilgalys \& Hester 1990), the ITS region with V9G (De Hoog \& Gerrits van den Ende 1998) and ITS4 (White et al. 1990), the GAPDH region with gpd1 and gpd2 (Berbee et al. 1999), the RPB2 region with RPB2-5F2 (Sung et al. 2007) and fRPB2-7cR (Liu et al. 1999) and the TEF1 gene with the primers EF1-728F and EF1986R (Carbone \& Kohn 1999) or EF2 (O’Donnell et al. 1998). The PCRs were performed in a MyCycler ${ }^{\mathrm{TM}}$ Thermal Cycler (Bio-Rad Laboratories B.V., Veenendaal, The Netherlands) in a total volume of $12.5 \mu \mathrm{~L}$. The SSU and LSU PCR mixtures consisted of $1 \mu \mathrm{~L}$ genomic DNA, $1 \times$ GoTaq® Flexi buffer (Promega, Madison, WI, USA), $2 \mu \mathrm{M} \mathrm{MgCl}_{2}, 40 \mu \mathrm{M}$ of each dNTP, $0.2 \mu \mathrm{M}$ of each primer and 0.25 Unit GoTaq® Flexi DNA polymerase (Promega). The ITS and GAPDH PCR mixtures differed from the original mix by containing $1 \mu \mathrm{M} \mathrm{MgCl}_{2}$, the RPB2 and TEF1 PCR mixtures differed from the original mix by containing $2 \mu \mathrm{~L}$ genomic DNA and the RPB2 mixture differed from the original mix by containing 0.5 U instead of 0.25 U GoTaq ${ }^{\circledR}$ Flexi DNA polymerase. Conditions for PCR amplification consisted of an initial denaturation step of 5 min at $94^{\circ} \mathrm{C}$ followed by 35 cycles of 30 s at $94^{\circ} \mathrm{C}, 30 \mathrm{~s}$ at $48^{\circ} \mathrm{C}$ and 90 s at $72^{\circ} \mathrm{C}$ for SSU, LSU, ITS and 40 cycles of 30 s at $94^{\circ} \mathrm{C}, 30 \mathrm{~s}$ at $52^{\circ} \mathrm{C} / 59^{\circ} \mathrm{C}$ and 45 s at $72^{\circ} \mathrm{C}$ for TEF1 using respectively EF2 or EF1-986R as reverse primer and a final elongation step of 7 min at $72^{\circ} \mathrm{C}$. The partial RPB2 gene was obtained by using a touchdown PCR protocol of 5 cycles of 45 s at $94^{\circ} \mathrm{C}, 45 \mathrm{~s}$ at $60^{\circ} \mathrm{C}$ and 2 min at $72^{\circ} \mathrm{C}$, followed by 5 cycles with a $58^{\circ} \mathrm{C}$ annealing temperature and 30 cycles with a $54^{\circ} \mathrm{C}$ annealing temperature. The PCR products were sequenced in both directions using the PCR primers and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's recommendations, and analysed with an ABI Prism 3730XL Sequencer (Applied Biosystems) according to the manufacturer's instructions. Consensus sequences were computed from forward and reverse sequences using the BioNumerics v. 4.61 software package (Applied Maths, St-Martens-Latem, Belgium). All generated sequences were deposited in GenBank (Table 1).

## Phylogenetic analyses

Multiple sequence alignments were generated with MAFFT v. 6.864b (http://mafft.cbrc.jp/ alignment/server/index.html), and adjusted by eye. Two different datasets were used to estimate two phylogenies; an Alternaria complex phylogeny and a Pleosporineae family tree. The first tree focusses on the Alternaria complex, the second one was produced to place the genera Comoclathris, Clathrospora and Alternariaster in the context of the Alternaria complex. The relatives of the three genera were determined with standard nucleotide blast searches, with both the SSU and LSU sequences, against the nucleotide database in GenBank. This resulted in a selection of 35 species (Table 1) for which the SSU, LSU and RPB2 sequence data set was present or could be completed. Blast searches with Embellisia annulata gave hits with two marine Dendryphiella species, Dendryphiella arenariae and Dendryphiella salina, which we also included. Phylogenetic analyses of the sequence data consisted of Bayesian and Maximum Likelihood analyses of both the individual data partitions as well as the combined aligned dataset. Bayesian analyses were performed with MrBayes v. 3.2.1 (Huelsenbeck \& Ronquist 2001, Ronquist \& Huelsenbeck 2003). The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The sample frequency was set at 100 and the temperature value of the heated chain was 0.1 . The temperature value was lowered to 0.05 when
Table 1. Isolates used in this study and their GenBank accession numbers. Bold accession numbers were generated in other studies

| Old species name | New species name | Alternaria section | Strain number ${ }^{1}$ | Status ${ }^{2}$ | Host / <br> Substrate | Country | Other collection ${ }^{1}$ | GenBank accession numbers |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  | SSU | LSU | RPB2 | ITS | GAPDH | TEF1 |
| Alternaria alternantherae | Alternaria alternantherae | Althernantherae | CBS 124392 |  | Solanum melongena | China | HSAUP2798 | KC584506 | KC584251 | KC584374 | KC584179 | KC584096 | KC584633 |
| Alternaria alternata | Alternaria alternata | Alternaria | CBS 916.96 | T | Arachis hypogaea | India | EGS 34.016 | KC584507 | DQ678082 | KC584375 | AF347031 | AY278808 | KC584634 |
| Alternaria anigozanthi | Alternaria anigozanthi | Eureka | CBS 121920 | T | Anigozanthus sp. | Australia | EGS 44.066 | KC584508 | KC584252 | KC584376 | KC584180 | KC584097 | KC584635 |
| Alternaria arborescens | Alternaria arborescens | Alternata | CBS 102605 | T | Lycopersicon esculentum | USA | EGS 39.128 | KC584509 | KC584253 | KC584377 | AF347033 | AY278810 | KC584636 |
| Alternaria argyranthemi | Alternaria argyranthemi |  | CBS 116530 | T | Argyranthemum sp. | New <br> Zealand | EGS 44.033 | KC584510 | KC584254 | KC584378 | KC584181 | KC584098 | KC584637 |
| Alternaria armoraciae | Alternaria armoraciae | Chalastospora | CBS 118702 | T | Armoracia rusticana | New <br> Zealand | EGS 51.064 | KC584511 | KC584255 | KC584379 | KC584182 | KC584099 | KC584638 |
| Alternaria avenicola | Alternaria avenicola | Panax | CBS 121459 | T | Avena sp. | Norway | EGS 50.185 | KC584512 | KC584256 | KC584380 | KC584183 | KC584100 | KC584639 |
| Alternaria axiaeriisporifera | Alternaria axiaeriisporifera | Gypsophilae | CBS 118715 | T | Gypsophila paniculata | New <br> Zealand | EGS 51.066 | KC584513 | KC584257 | KC584381 | KC584184 | KC584101 | KC584640 |
| Alternaria brassicae | Alternaria brassicae |  | CBS 116528 | R | Brassica oleracea | USA | EGS 38.032 | KC584514 | KC584258 | KC584382 | KC584185 | KC584102 | KC584641 |
| Alternaria brassicicola | Alternaria brassicicola | Brassicicola | CBS 118699 | R | Brassica oleracea | USA | EGS 42.002; <br> ATCC 96836 | KC584515 | KC584259 | KC584383 | JX499031 | KC584103 | KC584642 |
| Alternaria calycipyricola | Alternaria calycipyricola | Panax | CBS 121545 | T | Pyrus communis | China | $\begin{aligned} & \text { EGS 52.071; } \\ & \text { RGR } 96.0209 \end{aligned}$ | KC584516 | KC584260 | KC584384 | KC584186 | KC584104 | KC584643 |
| Alternaria capsicianпиі | Alternaria capsiciаппиі | Ulocladium | CBS 504.74 |  | Capsicum annuum | Unknown |  | KC584517 | KC584261 | KC584385 | KC584187 | KC584105 | KC584644 |
| Alternaria carotiincultae | Alternaria carotiincultae | Radicina | CBS 109381 | T | Daucus carota | USA | EGS 26.010 | KC584518 | KC584262 | KC584386 | KC584188 | KC584106 | KC584645 |
| Alternaria cheiranthi | Alternaria cheiranthi | Cheiranthus | CBS 109384 | R | Cheiranthus cheiri | Italy | EGS 41.188 | KC584519 | KC584263 | KC584387 | AF229457 | KC584107 | KC584646 |
| Alternaria chlamydospora | Alternaria chlamydospora | Phragmosporae | CBS 491.72 | T | Soil | Egypt | EGS 31.060; ATCC 28045; IMI 156427 | KC584520 | KC584264 | KC584388 | KC584189 | KC584108 | KC584647 |
| Alternaria cinerariae | Alternaria cinerariae | Sonchi | CBS 116495 | R | Ligularia sp. | USA | EGS 49.102 | KC584521 | KC584265 | KC584389 | KC584190 | KC584109 | KC584648 |
| Alternaria conjuncta | Alternaria conjuncta | Infectoriae | CBS 196.86 | T | Pastinaca sativa | Switzerland | EGS 37.139 | KC584522 | KC584266 | KC584390 | FJ266475 | AY562401 | KC584649 |
| Alternaria cumini | Alternaria cumini | Eureka | CBS 121329 | T | Cuminum cyminum | India | EGS 04.158a | KC584523 | KC584267 | KC584391 | KC584191 | KC584110 | KC584650 |
| Alternaria dauci | Alternaria dauci | Porri | CBS 117097 | R | Daucus carota | USA | EGS 46.006 | KC584524 | KC584268 | KC584392 | KC584192 | KC584111 | KC584651 |
| Alternaria daucifolii | Alternaria daucifolii | Alternaria | CBS 118812 | T | Daucus carota | USA | EGS 37.050 | KC584525 | KC584269 | KC584393 | KC584193 | KC584112 | KC584652 |
| Alternaria dianthicola | Alternaria dianthicola | Dianthicola | CBS 116491 | R | Dianthus $\times$ allwoodii | New <br> Zealand | EGS 51.022 | KC584526 | KC584270 | KC584394 | KC584194 | KC584113 | KC584653 |
| Alternaria elegans | Alternaria elegans | Dianthicola | CBS 109159 | T | Lycopersicon esculentum | Burkina <br> Faso | $\begin{aligned} & \text { EGS 45.072; } \\ & \text { IMI 374542 } \end{aligned}$ | KC584527 | KC584271 | KC584395 | KC584195 | KC584114 | KC584654 |
| Alternaria ellipsoidea | Alternaria ellipsoidea | Gypsophilae | CBS 119674 | T | Dianthus barbatus | USA | EGS 49.104 | KC584528 | KC584272 | KC584396 | KC584196 | KC584115 | KC584655 |


| Old species name | New species name | Alternaria section | Strain number ${ }^{1}$ | Status ${ }^{2}$ | Host / <br> Substrate | Country | Other collection ${ }^{1}$ | GenBank accession numbers |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  | SSU | LSU | RPB2 | ITS | GAPDH | TEF1 |
| Alternaria eryngii | Alternaria eryngii | Panax | CBS 121339 | R | Eryngium sp. | Unknown | EGS 41.005 | KC584529 | KC584273 | KC584397 | JQ693661 | AY562416 | KC584656 |
| Alternaria ethzedia | Alternaria ethzedia | Infectoriae | CBS 197.86 | T | Brassica napus | Switzerland | EGS 37.143 | KC584530 | KC584274 | KC584398 | AF392987 | AY278795 | KC584657 |
| Alternaria gaisen | Alternaria gaisen | Alternaria | CBS 632.93 | R | Pyrus pyrifolia cv. Nijiseiki | Japan | EGS 90.512 | KC584531 | KC584275 | KC584399 | KC584197 | KC584116 | KC584658 |
| Alternaria geniostomatis | Alternaria geniostomatis | Eureka | CBS 118701 | T | Geniostoma sp. | New <br> Zealand | EGS 51.061 | KC584532 | KC584276 | KC584400 | KC584198 | KC584117 | KC584659 |
| Alternaria gypsophilae | Alternaria gypsophilae | Gypsophilae | CBS 107.41 | T | Gypsophila elegans | Unknown | $\begin{aligned} & \text { EGS 07.025; } \\ & \text { IMI } 264349 \end{aligned}$ | KC584533 | KC584277 | KC584401 | KC584199 | KC584118 | KC584660 |
| Alternaria helianthiinficiens | Alternaria helianthiinficiens |  | CBS 117370 | R | Helianthus annuus | UK | EGS 50.174; <br> IMI 388636 | KC584534 | KC584278 | KC584402 | KC584200 | KC584119 | KC584661 |
| Alternaria helianthiinficiens | Alternaria helianthiinficiens |  | CBS 208.86 | T | Helianthus annuus | USA | EGS 36.184 | KC584535 | KC584279 | KC584403 | JX101649 | KC584120 | EU130548 |
| Alternaria infectoria | Alternaria infectoria | Infectoriae | CBS 210.86 | T | Triticum aestivum | UK | EGS 27.193 | KC584536 | KC584280 | KC584404 | DQ323697 | AY278793 | KC584662 |
| Alternaria japonica | Alternaria japonica | Japonicae | CBS 118390 | R | Brassica chinensis | USA | EGS 50.099 | KC584537 | KC584281 | KC584405 | KC584201 | KC584121 | KC584663 |
| Alternaria juxtiseptata | Alternaria juxtiseptata | Gypsophilae | CBS 119673 | T | Gypsophila paniculata | Australia | EGS 44.015; <br> DAR 43414 | KC584538 | KC584282 | KC584406 | KC584202 | KC584122 | KC584664 |
| Alternaria limaciformis | Alternaria limaciformis | Phragmosporae | CBS 481.81 | T | Soil | UK | EGS 07.086; IMI 052976; QM 1790 | KC584539 | KC584283 | KC584407 | KC584203 | KC584123 | KC584665 |
| Alternaria limoniasperae | Alternaria limoniasperae | Alternaria | CBS 102595 | T | Citrus jambhiri | USA | EGS 45.100 | KC584540 | KC584284 | KC584408 | FJ266476 | AY562411 | KC584666 |
| Alternaria longipes | Alternaria longipes | Alternaria | CBS 540.94 | R | Nicotiana tabacum | USA | $\begin{aligned} & \text { EGS 30.033; } \\ & \text { QM } 9589 \end{aligned}$ | KC584541 | KC584285 | KC584409 | AY278835 | AY278811 | KC584667 |
| Alternaria macrospora | Alternaria macrospora | Porri | CBS 117228 | T | Gossypium barbadense | USA | EGS 50.190 | KC584542 | KC584286 | KC584410 | KC584204 | KC584124 | KC584668 |
| Alternaria mimicula | Alternaria mimicula | Brassicicola | CBS 118696 | T | Lycopersicon esculentum | USA | $\begin{aligned} & \text { EGS 01.056; } \\ & \text { QM 26a } \end{aligned}$ | KC584543 | KC584287 | KC584411 | FJ266477 | AY562415 | KC584669 |
| Alternaria molesta | Alternaria molesta | Phragmosporae | CBS 548.81 | T | Phocaena phocaena | Denmark | EGS 32.075 | KC584544 | KC584288 | KC584412 | KC584205 | KC584125 | KC584670 |
| Alternaria mouchaccae | Alternaria mouchaccae | Phragmosporae | CBS 119671 | T | Soil | Egypt | EGS 31.061 | KC584545 | KC584289 | KC584413 | KC584206 | AY562399 | KC584671 |
| Alternaria nepalensis | Alternaria nepalensis | Japonicae | CBS 118700 | T | Brassica sp. | Nepal | $\begin{aligned} & \text { EGS 45.073; } \\ & \text { IMI } 374543 \end{aligned}$ | KC584546 | KC584290 | KC584414 | KC584207 | KC584126 | KC584672 |
| Alternaria nobilis | Alternaria nobilis | Gypsophilae | CBS 116490 | R | Dianthus caryophyllus | New <br> Zealand | EGS 51.027; NZMAF Lynfield 743 | KC584547 | KC584291 | KC584415 | KC584208 | KC584127 | KC584673 |
| Alternaria oregonensis | Alternaria oregonensis | Infectoriae | CBS 542.94 | T | Triticum aestivum | USA | EGS 29.194 | KC584548 | KC584292 | KC584416 | FJ266478 | FJ266491 | KC584674 |
| Alternaria panax | Alternaria panax | Panax | CBS 482.81 | R | Aralia racemosa | USA | EGS 29.180 | KC584549 | KC584293 | KC584417 | KC584209 | KC584128 | KC584675 |
| Alternaria perpunctulata | Alternaria perpunctulata | Althernantherae | CBS 115267 | T | Alternanthera philoxeroides | USA |  | KC584550 | KC584294 | KC584418 | KC584210 | KC584129 | KC584676 |
| Alternaria petroselini | Alternaria petroselini | Radicina | CBS 112.41 | T | Petroselinum sativum | Unknown | EGS 06.196 | KC584551 | KC584295 | KC584419 | KC584211 | KC584130 | KC584677 |

Table 1．（Continued）． Old species New species Alternaria name name section Alternaria photistica Alternaria photistica Panax Alternaria porri Alternaria porri Porri Alternaria Alternaria $\begin{array}{ll}\text { pseudorostrata } & \text { pseudorostrata } \\ \text { Alternaria radicina } & \text { Alternaria radicina }\end{array}$
CBS 115.44 CBS 116492 CBS 109382 CBS 106.41
CBS 115265
CBS 109380 CBS 116651 CBS 118387
CBS 118698 CBS 119675 CBS 479.81
CBS 918.96 CBS 121712 CBS 119676 CBS 116533 CBS 118714 No
O
थै
थै $\begin{aligned} & \text { Other } \\ & \text { collection }\end{aligned}$
EGS 35.172
EGS 48.147
EGS 42.060

EGS 03．145；
ATCC 6503；
IMI 124939；
QM 1301；QM
6503 EGS 07.030

 EGS 52.089 ，
MUCL 20298

 EGS 33.024 io
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0 IMI 049788 $\stackrel{\rightharpoonup}{0}$
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 EGS 41.070 $\infty$
$\underset{y}{\infty}$
$\underset{y}{+}$
H EGS 46．003； ATCC 26038
EGS 36.007

 Netherlands $\begin{array}{ll}\text { Sesamum indicum } & \text { Argentina } \\ \text { Smyrnium } & \text { UK }\end{array}$ USA岁
皆 Germany坒岂氐岕 Host／
Substrate Digitalis purpu Euphorbia pulcherrima
Daucus carota Reseda odorata
$\begin{aligned} & \text { Saponaria } \\ & \text { officinalis }\end{aligned}$ Petroselinum crispum crispum
Reseda odorata Strain number $^{1}$ Status $^{2}$ CBS 212.86 T CBS 116698 CBS 119411 CBS 245.67 $\approx$ T olusatrum Solanum
tuberosum Soil Sonchus asper Tagetes erecta Thalictrum sp． Triglochin
 Vaccaria
hispanica Vaccaria hispanica Helianthus sp． EGS 42．060 KC584554

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KC584299 KC584423 KC584213 KC584133 EGS 07．030 KC584556 KC584300 KC584424 KC584214 KC584134 KC584557 KC584301 KC584425 KC584215 KC584135 KC584558 KC584302 KC584426 AF229455 AY278800 KC584559 KC584303 KC584427 KC584216 KC584136 KC584560 $\quad$ KC584304 $\quad$ KC584428 $\quad$ JF780937 $\quad$ KC584137 KC584561 KC584305 KC584429 AF229456 KC584138 KC584562 KC584306 KC584430 KC584217 KC584139 KC584563 KC584307 KC584431 KC584218 KC584140 KC584564 KC584308 KC584432 KC584219 KC584141 KC584565 KC584309 KC584433 KC584220 KC584142 KC584143跲 KC584144 $\stackrel{\text { 夺 }}{\ddagger}$ KC584146 C584221 EU040211 KC584222 त
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 C584434 KC584436 | 气 |
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Table 1. (Continued).
Old species New species Alternaria
Alternaria
section
Strain
number
CBS 327.69
CBS 126.54
CBS 116606
CBS 116608

CBS 216.75
CBS 121340
CBS 121331

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CBS 567.66
CBS 161.51

CBS 134.39 DAOM 226212 CBS 174.52 $\begin{array}{lll}\text { CBS } 156.53 & \text { Castilleja miniata } & \text { USA } \\ \text { CBS 157.53 } & \begin{array}{l}\text { Ligusticum } \\ \text { purpureum }\end{array} & \text { USA } \\ \text { CBS 400.71 } & \begin{array}{l}\text { Chamaerops }\end{array} & \text { Italy }\end{array}$ $\begin{array}{lll}\text { CBS } 156.53 & \text { Castilleja miniata } & \text { USA } \\ \text { CBS 157.53 } & \begin{array}{l}\text { Ligusticum } \\ \text { purpureum }\end{array} & \text { USA } \\ \text { CBS 400.71 } & \begin{array}{l}\text { Chamaerops }\end{array} & \text { Italy }\end{array}$ $\begin{array}{lll}\text { CBS 156.53 } & \text { Castilleja miniata } & \text { USA } \\ \text { CBS 157.53 } & \begin{array}{l}\text { Ligusticum } \\ \text { purpureum }\end{array} & \text { USA } \\ \text { CBS 400.71 } & \begin{array}{l}\text { Chamaerops }\end{array} & \text { Italy }\end{array}$
 Other
collection KC584627 KC584369 KC584494
EU754038 DQ678070 DQ677967 EU754084 EU754183 GU371780 KC584579 KC584321 KC584446 KC584572 KC584316 KC584440 DQ678001 DQ678054 KC584499
EU754045 EU754144 GU371777 KC584573 KC584317 KC584441 KC584574 KC584318 KC584442 KC584575 FJ839651 KC584443 KC584576 KC584319 KC584444 KC584628 KC584370 KC584495 KC584629 KC584371 KC584496 KC584577 KC584320 KC584445 AY544727 AY544645 DQ247790 DQ677995 DQ678045 DQ677939 KC584578 DQ678068 DQ677964 KC584630 KC584372 KC584497 KC584631 KC584373 KC584498
EU754054 EU754153 DQ677956

 1633 DAOM 230456 Country Country Unknown
Netherlands
Netherlands Uetherland Austri Netherlands Germany Australia Australia USA Slovakia
Switzerland
Switzerland
USA Unknown Canada $\begin{array}{ll}\text { Hordeum vulgare } & \text { Canada } \\ \begin{array}{l}\text { Anemone } \\ \text { occidentalis }\end{array} & \text { USA }\end{array}$ Papaver rhoeas Austria Host /
Substrate Helianthus annuus Pisum sativum Solanum tuberosum Papaver Papaver rhoeas Halimione portulacoides Anthyllis
vulneraria vulneraria
Elymus scabrus Triticum sp. e Human E
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0
0
0 Carex curvula Juncus
mertensianus Zea mays Hordeum vulgare Castilleja purpureum Chamaerop Status ${ }^{2}$
 $\begin{array}{ll}\text { Old species } & \text { New species } \\ \text { name } & \text { name }\end{array}$ Alternariaster Alternariaster Alternariaster
helianthi
Ascochyta pisi Asco
Boeremia exigua
$\begin{array}{ll}\text { Ascochyta pisi } & \text { Ascochyta pisi } \\ \text { Borma }\end{array}$




$\qquad$ hispidulum
Alternaria cetera Alternaria
breviramosa Alternaria obclavata Alternaria slovaca
Clathrospora elynae
Clathrospora elynae
Alternaria sp. Cochliobolus Cochliobolus
heterostrophus Cochliobolus sativus Cochliobolus sativus Comoclathris magna Alternaria sp .

| Comoclathris | Comoclathris |
| :--- | :--- |
| compressa | compressa |
| Comoclathris | Comoclathris |
| compressa | compressa |
| Coniothyrium | Coniothyrium <br> palmarum |
| palmarum <br> Crivellia <br> papaveracea | Alternaria <br> penicilata |

Table 1. (Continued).
Country Other
collection ${ }^{1}$
DAOM 63738;
IMI 067735;
MUCL 4129
MUCL 9639
EGS 29.159
ATCC 22412;
IMI 155707;
MUCL 18571;
QM 8609
EGS 10.073;
ATCC 22409;
IMI 155709;
MUCL 18573;
QM 7287
KC584585 $\quad$ KC584327 $\quad$ KC584452 $\quad$ AF348226 $\quad$ FJ348227 $\quad$ KC584711




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KC584597 $\quad$ KC584339 $\quad$ KC584464 $\quad$ AY278842 $\quad$ KC584161 $\quad$ KC584723 QM
 EGS 39.099
EGS 27.098;
ATCC 18914
IMI 115034
IMI 320290;
IMI 341684
France
UK
New
Zealand Zealand
苋
North Sea,
Skagerrak USA
 Adriatic Sea Australia
Netherlands USA
 Zealand New
Zealand

 Ricinus communis Senecio jacobaea Senecio jacobaea Seawater Medicago rugosa Hyacinthus Septinella dioica Lolium perenne
 Soil
 Protea sp. Status ${ }^{2}$ Host / Substrate Coastal sand Spartina sp. Fragaria sp. Allium sativum Cancer pagurus Air

$\qquad$
$\bumpeq$
 CBS 132.89 CBS 110533 CBS 476.90 CBS 766.79 CBS 193.86
CBS 416.71 CBS 536.83
CBS 477.90 CBS 115266 CBS 478.90
CBS 274.70 $\infty$
$\underset{\sim}{\infty}$
ஸै CBS 475.90 Strain
number CBS 181.58 CBS 142.60 CBS 534.83
CBS 339.71 CBS 302.84
CBS 341.71 Chalastospora Embellisia allii Alternaria embellisia Embellisia $\begin{array}{lll}\text { Old species } & \text { New species } & \begin{array}{l}\text { Alternaria } \\ \text { name }\end{array} \\ \text { name } & \text { section }\end{array}$ $\begin{array}{ll}\begin{array}{l}\text { Dendryphiella } \\ \text { arenariae }\end{array} & \begin{array}{l}\text { Paradendryphiella } \\ \text { arenariae }\end{array}\end{array}$
Dendryphiella salina Paradendryphiella salina Embellisia abundans Alternaria abundans
arella $\begin{array}{ll} & \text { salina } \\ \text { Embellisia } & \begin{array}{l}\text { Alternaria } \\ \text { chlamydosporigena }\end{array}\end{array}$ chlamydospora chlamydosporigena

| Embellisia conoidea | Alternaria conoidea | Brassicicola |
| :--- | :--- | :--- |
| Embellisia dennisii | Alternaria dennisii |  |


| Old species name | New species name | Alternaria section | Strain number ${ }^{1}$ | Status ${ }^{2}$ | Host / <br> Substrate | Country | Other collection ${ }^{1}$ | GenBank accession numbers |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  | SSU | LSU | RPB2 | ITS | GAPDH | TEF1 |
| Embellisia tellustris | Alternaria tellustris | Embellisia | CBS 538.83 | T | Soil | USA | EGS 33.026 | KC584598 | KC584340 | KC584465 | FJ357316 | AY562419 | KC584724 |
| Embellisia tumida | Alternaria tumida | Embellisioides | CBS 539.83 | T | Triticum aestivum | Australia |  | KC584599 | KC584341 | KC584466 | FJ266481 | FJ266493 | KC584725 |
| Heterospora chenopodii | Heterospora chenopodii |  | CBS 115.96 |  | Chenopodium album | Netherlands | PD 94/1576 | EU754089 | EU754188 | GU371775 |  |  |  |
| Julella avicenniae | Julella avicenniae |  | BCC 18422 |  | Mangrove wood | Thailand |  | GU371831 | GU371823 | GU371787 |  |  |  |
| Leptosphaerulina australis | Leptosphaerulina australis |  | CBS 317.83 |  | Eugenia aromatica | Indonesia |  | GU296160 | GU301830 | GU371790 |  |  |  |
| Loratospora aestuarii | Loratospora aestuarii |  | JK 5535B |  | Juncus roemerianus | USA |  | GU296168 | GU301838 | GU371760 |  |  |  |
| Neophaeosphaeria filamentosa | Neophaeosphaeria filamentosa |  | CBS 102202 |  | Yucca rostrata | Mexico |  | GQ387516 | GQ387577 | GU371773 |  |  |  |
| Nimbya caricis | Alternaria caricis | Nimbya | CBS 480.90 | T | Carex hoodii | USA | EGS 13.094 | KC584600 | KC584342 | KC584467 | AY278839 | AY278826 | KC584726 |
| "Nimbya gomphrenae" | Alternaria sp. | Alternaria | CBS 108.27 |  | Gomphrena globosa | Unknown |  | KC584601 | KC584343 | KC584468 | KC584236 | KC584162 | KC584727 |
| Nimbya scirpicola | Alternaria scirpicola | Nimbya | CBS 481.90 | R | Scirpus sp. | UK | EGS 19.042 | KC584602 | KC584344 | KC584469 | KC584237 | KC584163 | KC584728 |
| Ophiosphaerella herpotricha | Ophiosphaerella herpotricha |  | CBS 620.86 |  | Bromus erectus | Switzerland | ETH 9373 | DQ678010 | DQ678062 | DQ677958 |  |  |  |
| Paraleptosphaeria dryadis | Paraleptosphaeria dryadis |  | CBS 643.86 |  | Dryas octopetala | Switzerland | ETH 9446 | KC584632 | GU301828 | GU371733 |  |  |  |
| Peyronellaea glomerata | Peyronellaea glomerata |  | CBS 528.66 |  | Chrysanthemum sp. | Netherlands | PD 63/590 | EU754085 | EU754184 | GU371781 |  |  |  |
| Peyronellaea zeaemaydis | Peyronellaea zeaemaydis |  | CBS 588.69 | T | Zea mays | USA |  | EU754093 | EU754192 | GU371782 |  |  |  |
| Phaeosphaeria ammophilae | Phaeosphaeria ammophilae |  | CBS 114595 |  | Ammophila arenaria | Sweden | UPSC 3568 | GU296185 | GU304859 | GU371724 |  |  |  |
| Phaeosphaeria avenaria | Phaeosphaeria avenaria |  | DAOM 226215 |  | Avena sativa | Canada | OSC 100096 | AY544725 | AY544684 | DQ677941 |  |  |  |
| Phaeosphaeria eustoma | Phaeosphaeria eustoma |  | CBS 573.86 |  | Dactylis glomerata | Switzerland | ETH 9239 | DQ678011 | DQ678063 | DQ677959 |  |  |  |
| Phoma complanata | Phoma complanata |  | CBS 268.92 |  | Anglica sylvestris | Netherlands | PD 75/3 | EU754081 | EU754180 | GU371778 |  |  |  |
| Phoma herbarum | Phoma herbarum |  | CBS 276.37 |  | Wood pulp | Sweden |  | DQ678014 | DQ678066 | DQ677962 |  |  |  |
| Plenodomus lingam | Plenodomus lingam |  | DAOM 229267 |  | Brassica sp. | France |  | DQ470993 | DQ470946 | DQ470894 |  |  |  |
| Pleospora betae | Pleospora betae |  | CBS 109410 |  | Beta vulgaris | Netherlands | PD 77/113 | EU754079 | EU754178 | GU371774 |  |  |  |
| Pleospora calvescens | Pleospora calvescens |  | CBS 246.79 |  | Atriplex hastata | Germany | PD 77/655 | EU754032 | EU754131 | KC584500 |  |  |  |
| Pleospora chenopodii | Pleospora chenopodii |  | CBS 206.80 |  | Chenopodium quinoa | Bolivia | PD 74/1022 | JF740095 | JF740266 | KC584501 |  |  |  |
| Pleospora fallens | Pleospora fallens |  | CBS 161.78 |  | Olea europaea | New <br> Zealand |  | GU238215 | GU238074 | KC584502 |  |  |  |
| Pleospora halimiones | Pleospora halimiones |  | CBS 432.77 |  | Halimione portulacoides | Netherlands | IMI 282137 | JF740096 | JF740267 | KC584503 |  |  |  |


| Old species name | New species name | Alternaria section | Strain number ${ }^{1}$ | Status ${ }^{2}$ | Host / <br> Substrate | Country | Other collection ${ }^{1}$ | GenBank accession numbers |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  | SSU | LSU | RPB2 | ITS | GAPDH | TEF1 |
| Pleospora incompta | Pleospora incompta |  | CBS 467.76 |  | Olea europaea | Greece |  | GU23822 | GU238087 | KC584504 |  |  |  |
| Pleospora tarda | Pleospora tarda |  | CBS 714.68 | T | Medicago sativa | Canada | EGS 04.118C; IMI 135456; MUCL 11717; QM 1379 | KC584603 | KC584345 | AF107804 | KC584238 | AF443881 | KC584729 |
| Pleospora typhicola | Pleospora typhicola |  | CBS 132.69 |  | Typha angustifolia | Netherlands |  | JF740105 | JF740325 | KC584505 |  |  |  |
| Pyrenochaeta nobilis | Pyrenochaeta nobilis |  | CBS 407.76 | T | Laurus nobilis | Italy |  | EU754107 | DQ678096 | DQ677991 |  |  |  |
| Pyrenophora phaeocomes | Pyrenophora phaeocomes |  | DAOM 222769 |  | Calamagrostis villosa | Switzerland |  | DQ499595 | DQ499596 | DQ497614 |  |  |  |
| Saccothecium sepincola | Saccothecium sepincola |  | CBS 278.32 |  | Ribes nigrum | USA |  | GU296195 | GU301870 | GU371745 |  |  |  |
| Setomelanomma holmii | Setomelanomma holmii |  | CBS 110217 |  | Picea pungens | USA |  | GU296196 | GQ37633 | GU371800 |  |  |  |
| Sinomyces alternariae | Alternaria alternariae | Ulocladium | CBS 126989 | T | Daucus carota | USA | EGS 46.004 | KC584604 | KC584346 | KC584470 | AF229485 | AY278815 | KC584730 |
| Stemphylium herbarum | Stemphylium herbarum |  | CBS 191.86 | T | Medicago sativa | India | $\begin{aligned} & \text { EGS 36.138; } \\ & \text { IMI 276975 } \end{aligned}$ | GU238232 | GU238160 | KC584471 | KC584239 | AF443884 | KC584731 |
| Teretispora leucanthemi | Alternaria leucanthemi | Teretispora | CBS 421.65 | T | Chrysanthemum maximum | Netherlands | ATCC 16028; <br> IFO 9085; IMI <br> 111986; QM <br> 7227 | KC584605 | KC584347 | KC584472 | KC584240 | KC584164 | KC584732 |
| Teretispora leucanthemi | Alternaria leucanthemi | Teretispora | CBS 422.65 | R | Chrysanthemum maximum | USA | EGS 17.063; ATCC 16029; IMI 111987; QM 8579 | KC584606 | KC584348 | KC584473 | KC584241 | KC584165 | KC584733 |
| Ulocladium arborescens | Alternaria aspera | Pseudoulocladium | CBS 115269 | T | Pistacia vera | Japan | IMI 369777 | KC584607 | KC584349 | KC584474 | KC584242 | KC584166 | KC584734 |
| Ulocladium atrum | Alternaria atra | Ulocladioides | CBS 195.67 | T | Soil | USA | ATCC 18040; IMI 124944; QM 8408 | KC584608 | KC584350 | KC584475 | AF229486 | KC584167 | KC584735 |
| Ulocladium botrytis | Alternaria botrytis | Ulocladium | CBS 197.67 | T | Contaminant | USA | ATCC 18042; <br> IMI 124942; MUCL 18556; QM 7878 | KC584609 | KC584351 | KC584476 | KC584243 | KC584168 | KC584736 |
| Ulocladium botrytis | Alternaria sp. | Ulocladioides | CBS 198.67 | R | Soil | USA | ATCC 18043; IMI 124949; MUCL 18557; QM 8619 | KC584610 | KC584352 | KC584477 | AF229487 | KC584169 | KC584737 |
| Ulocladium brassicae | Alternaria <br> brassicae-pekinensis | Ulocladioides | CBS 121493 | T | Brassica pekinensis | China | HSAUPwy0037 | KC584611 | KC584353 | KC584478 | KC584244 | KC584170 | KC584738 |


| Old species name | New species name | Alternaria section | Strain number ${ }^{1}$ | Status ${ }^{2}$ | Host / <br> Substrate | Country | Other collection ${ }^{1}$ | GenBank accession numbers |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |  |  | SSU | LSU | RPB2 | ITS | GAPDH | TEF1 |
| Ulocladium cantlous | Alternaria cantlous | Ulocladioides | CBS 123007 | T | Cucumis melo | China | HSAUP0209 | KC584612 | KC584354 | KC584479 | KC584245 | KC584171 | KC584739 |
| Ulocladium capsici | Alternaria concatenata | Pseudoulocladium | CBS 120006 | T | Unknown | Unknown | HSAUPIII 0035 | KC584613 | KC584355 | KC584480 | KC584246 | AY762950 | KC584740 |
| Ulocladium chartarum | Alternaria chartarum | Pseudoulocladium | CBS 200.67 | T | Populus sp. | Canada | ATCC 18044; DAOM 59616b; IMI 124943; MUCL 18564; QM 8328 | KC584614 | KC584356 | KC584481 | AF229488 | KC584172 | KC584741 |
| Ulocladium consortiale | Alternaria consortialis | Ulocladioides | CBS 104.31 | T | Unknown | Unknown |  | KC584615 | KC584357 | KC584482 | KC584247 | KC584173 | KC584742 |
| Ulocladium cucurbitae | Alternaria cucurbitae | Ulocladioides | CBS 483.81 | R | Cucumis sativus | New <br> Zealand | EGS 31.021; <br> LEV 7067 | KC584616 | KC584358 | KC584483 | FJ266483 | AY562418 | KC584743 |
| Ulocladium multiforme | Alternaria multiformis | Ulocladioides | CBS 102060 | T | Soil | Canada |  | KC584617 | KC584359 | KC584484 | FJ266486 | KC584174 | KC584744 |
| Ulocladium obovoideum | Alternaria obovoidea | Ulocladioides | CBS 101229 |  | Cucumis sativus | New <br> Zealand |  | KC584618 | KC584360 | KC584485 | FJ266487 | FJ266498 | KC584745 |
| Ulocladium oudemansii | Alternaria oudemansii | Ulocladium | CBS 114.07 | T | Unknown | Unknown | ATCC 18047; <br> IMI 124940; MUCL 18563; QM 1744 | KC584619 | KC584361 | KC584486 | FJ266488 | KC584175 | KC584746 |
| Ulocladium septosporum | Alternaria septospora | Pseudoulocladium | CBS 109.38 |  | Wood | Italy |  | KC584620 | KC584362 | KC584487 | FJ266489 | FJ266500 | KC584747 |
| Ulocladium solani | Alternaria heterospora | Ulocladioides | CBS 123376 | T | Lycopersicon esculentum | China | HSAUP 0521 | KC584621 | KC584363 | KC584488 | KC584248 | KC584176 | KC584748 |
| Ulocladium subcucurbitae | Alternaria subcucurbitae | Ulocladioides | CBS 121491 | T | Chenopodium glaucum | China |  | KC584622 | KC584364 | KC584489 | KC584249 | EU855803 | KC584749 |
| Ulocladium tuberculatum | Alternaria terricola | Ulocladioides | CBS 202.67 | T | Soil | USA | ATCC 18048; <br> IMI 124947; <br> MUCL 18560; <br> QM 8614 | KC584623 | KC584365 | KC584490 | FJ266490 | KC584177 | KC584750 |
| Undifilum bornmuelleri | Alternaria bornmuelleri | Undifilum | DAOM 231361 |  | Securigera varia | Austria | DAOM 231361 | KC584624 | KC584366 | KC584491 | FJ357317 | FJ357305 | KC584751 |
| Ybotromyces caespitosus | Alternaria caespitosa | Infectoriae | CBS 177.80 | T | Human | Spain |  | KC584625 | KC584367 | KC584492 | KC584250 | KC584178 | KC584752 |






 Culture Collection, Amherst, MA, USA.
${ }^{2}$ T: ex-type strain; R: representative strain
Table 2. Summary of locus and phylogenetic results as well as a heat map of the Bayesian posterior probabilities and RAxML boostrap support values per Alternaria section.

|  | 1-region |  |  |  |  |  | 2-region |  |  | 3-region | 6-region <br> SSU | 1-region |  |  |  |  |  | 2-region |  |  | 3-region | 6-region |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | SSU | LSU | ITS | GAPDH | RPB2 | TEF1 | $\begin{gathered} \text { GAPDH } \\ \text { RPB2 } \end{gathered}$ | $\begin{gathered} \text { GAPDH } \\ \text { TEF1 } \end{gathered}$ | $\begin{gathered} \text { RPB2 } \\ \text { TEF1 } \end{gathered}$ | GAPDH <br> RPB2 <br> TEF1 | SSU <br> LSU <br> ITS <br> GAPDH <br> RPB2 <br> TEF1 | SSU | LSU | ITS | GAPDH | RPB2 | TEF1 | $\begin{array}{\|c\|} \hline \text { GAPDH } \\ \text { RPB2 } \end{array}$ | $\begin{gathered} \text { GAPDH } \\ \text { TEF1 } \end{gathered}$ | RPB2 TEF1 | GAPDH <br> RPB2 <br> TEF1 | $\begin{gathered} \text { SSU } \\ \text { LSU } \\ \text { ITS } \\ \text { GAPDH } \\ \text { RPB2 } \\ \text { TEF1 } \end{gathered}$ |
| Aligned length | 1021 | 851 | 499 | 573 | 786 | 269 | 1359 | 842 | 1055 | 1628 | 3999 | 1021 | 851 | 499 | 573 | 786 | 269 | 1359 | 842 | 1055 | 1628 | 3999 |
| Unique site patterns | 45 | 57 | 148 | 272 | 296 | 224 | 568 | 496 | 520 | 792 | 1042 | 45 | 57 | 148 | 272 | 296 | 224 | 568 | 496 | 520 | 792 | 1042 |
| No. of sampled trees (post burnin) | 39002 | 31578 | 75002 | 23702 | 56028 | 12452 | 10128 | 13728 | 44852 | 5778 | 16278 |  |  |  |  |  |  |  |  |  |  |  |
|  | Bayesian Posterior Probabilities |  |  |  |  |  |  |  |  |  |  | RAxML bootstrap support |  |  |  |  |  |  |  |  |  |  |
| Sect. Alternantherae |  | * |  |  |  |  |  |  |  |  |  |  | * |  |  |  |  |  |  |  |  |  |
| Sect. Alternaria |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Chalastospora * |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Crivellia |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Embellisia |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Embellisioides |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Gypsophilae |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Infectoriae |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Japonicae |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Nimbya |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. panax |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Pseudoulocladium |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Radicina |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Sonchi |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Teretispora |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sect. Ulocladioides <br> Sect. Ulocladium | * | * |  |  |  |  |  |  |  |  |  | * | * |  |  |  |  |  |  |  |  |  |
|  |  |  | * |  |  |  |  |  |  |  |  |  |  | * |  |  |  |  |  |  |  |  |

the average standard deviation of split frequencies did not fall below 0.01 after 5 M generations (RPB2 and Pleosporineae phylogeny). Burn-in was set to $25 \%$ after which the likelihood values were stationary. Maximum likelihood analyses including 500 bootstrap replicates were run using RAxML v. 7.2.6 (Stamatakis \& Alachiotis 2010). The online tool Findmodel (http://www.hiv. lanl.gov/content/sequence/findmodel/findmodel.html) was used to determine the best nucleotide substitution model for each partition. For the SSU (Pleosporineae family tree), LSU, ITS, RPB2 and TEF1 partitions a GTR model with a gamma-distributed rate variation was suggested, and for the SSU (Alternaria complex) and GAPDH partitions a TrN model with gamma-distributed rate variation. Sequences of Stemphylium herbarum (CBS 191.86) were used as outgroup in the Alternaria phylogeny and those of Jullella avenicae (BCC 18422) in the Pleosporineae phylogeny. The resulting trees were printed with TreeView v. 1.6.6 (Page 1996) and together with the alignments deposited into TreeBASE (http://www.treebase.org).

## RESULTS

## Phylogeny

For defining the taxonomy of Alternaria and allied genera, 121 strains were included in the Alternaria complex alignment. The alignment length and unique site patterns of the different genes and gene combinations are stated in Table 2. The original ITS alignment consisted of 577 characters of which the first 78 are excluded as this contained a non-alignable region. In the original TEF1 alignment ( 375 characters) we coded the major inserts (Table 3), which otherwise would negatively influence the phylogeny, resulting in a TEF1 alignment of 269 characters. All phylogenies, different phylogenetic methods and gene regions or gene combinations used on this dataset (data not shown, trees and alignments lodged in TreeBASE), show a weak support at the deeper nodes of the tree. The only well-supported node (Bayesian posterior probability of 1.0, RAxML Maximum Likelihood support value of 100) in all phylogenies separates Embellisia annulata CBS 302.84 and the Pleospora / Stemphylium clade from the Alternaria complex (Fig. 1). In the Alternaria clade, six monotypic lineages and 24 internal clades occur consistently in the individual and combined phylogenies, although positions vary between the different gene regions or combinations used. The support values for the clades within Alternaria (called sections) are plotted in a heat map (Table 2) per gene and phylogenetic method used. The support values for the different phylogenetic methods vary, with the Bayesian posterior probabilities being higher than the RAxML bootstrap support values (Table 2). The SSU, LSU and ITS phylogenies display a low resolution, which reflects in poor to no support of the sections. Therefore, we chose not to include them in the multi-gene alignments, except in the all-gene alignment. In the GAPDH phylogenies, sect. Cheiranthus, sect. Nimbya and sect. Pseudoulocladium are poorly supported and "A. resedae" clusters separate from sect. Cheiranthus. In the RPB2 phylogenies the support values for sect. Alternaria, sect. Embellisioides and sect. Eureka are relatively low; A. cumini clusters in sect. Embellisioides instead of sect. Eureka and U. capsici clusters separate from sect. Pseudoulocladium. The TEF1 phylogenies did not support sect. Nimbya and show relative low support for sect. Cheiranthus, sect. Dianthicola, sect. Embellisioides, sect. Panax, sect. Phragmosporae and sect. Radicina, and A. cumini clusters outside sect. Eureka. In the 2-region phylogenies U. capsici clusters outside sect. Pseudoulocladium based on GAPDH and RPB2, E. indefessa clusters outside sect. Cheiranthus based on GAPDH and TEF1, and sect. Eureka is poorly supported based on RPB2 and TEF1. The combined phylogeny based on the GAPDH,


Fig. 1. Bayesian $50 \%$ majority rule consensus tree based on the GAPDH, RPB2 and TEF1 sequences of 121 strains representing the Alternaria complex. The Bayesian posterior probabilities (PP) and RAxML bootstrap support values (ML) are given at the nodes (PP/ML). Thickened lines indicate a PP of 1.0 and ML of 100. The tree was rooted to Stemphylium herbarum (CBS 191.86). The monotypic lineages are indicated by black dots.

Table 3. Coded inserts in the TEF1 sequence alignment.

| Species | Nt position | Coded | Nt position | Coded |
| :--- | :--- | :--- | :--- | :--- |
| Alternaria elegans | 23 to 39 | TC |  |  |
| Alternaria simsimi | 23 to 39 | TCC |  |  |
| Alternaria dauci | 186 to 205 | C | 221 to 269 | TACTT |
| Alternaria macrospora | 186 to 205 | C | 221 to 269 | TCCCC |
| Alternaria porri | 186 to 205 | C | 221 to 269 | ACTTA |
| Alternaria pseudorostrata | 186 to 205 | C | 221 to 269 | TGGTA |
| Alternaria solani | 186 to 205 | C | 221 to 269 | -AAGG |
| Alternaria tegetica | 186 to 205 | C | 221 to 269 | CACAC |

RPB2 and TEF1 sequences (Fig. 1) is displayed, as these are the genes with the best resolution.
The final Pleosporineae alignment included 74 strains, representing six families, and consisted of 2506 characters (SSU 935, LSU 796, RPB2 775) of which 700 were unique site patterns (SSU 111, LSU 145, RPB2 444). In the SSU alignment a large insertion at position 446 in the isolates Chaetosphaeronema hispidulum CBS 216.75, Pleospora fallens CBS 161.78, Pleospora flavigena CBS 314.80 and Ophiosphaerella herpotrichia CBS 620.86 was excluded from the phylogenetic analyses. A total of 43202 trees were sampled after the burn-in. The type species of Clathrospora, C. elynae, forms a well-supported clade, located basal to the Pleosporaceae (Fig. 2), outside the Alternaria complex. The type species of Comoclathris, C. lanata, was not available for study but the two Comoclathris compressa strains cluster in a wellsupported clade within the Pleosporaceae outside Alternaria s. str. The genus Alternariaster, with Alternariaster helianthi as type and only species, also clusters outside the Alternaria complex and even outside Pleosporaceae; it belongs to the Leptosphaeriaceae instead (Fig. 2). Embellisia annulata is identical to Dendryphiella salina, and forms a well-supported clade in the Pleosporaceae together with Dendryphiella arenariae. As the type species of Dendryphiella, D. vinosa, clusters outside the Pleosporineae (dela Cruz 2006, Jones et al. 2008), Dendryphiella salina and D. arenariae are placed in a new genus, Paradendryphiella, below.

## Taxonomy

Based on DNA sequence data in combination with a review of literature and morphology, the species within the Alternaria clade are all recognised here as Alternaria (Fig 1). This puts the genera Allewia, Brachycladium, Chalastospora, Chmelia, Crivellia, Embellisia, Lewia, Nimbya, Sinomyces, Teretispora, Ulocladium, Undifilum and Ybotromyces in synonymy with Alternaria, resulting in the proposal of 32 new combinations, 10 new names and the resurrection of 10 names. Species of Alternaria were assigned to 24 Alternaria sections, of which 16 are newly described, and six monotypic lineages. The (emended) description of the genus Alternaria, the Alternaria sections and monotypic lineages with new Alternaria names and name combinations are treated below in alphabetical order. Finally the description of the new genus Paradendryphiella is also provided.

Alternaria Nees, Syst. Pilze (Würzburg): 72. 1816 [1816-1817].
= Elosia Pers., Mycol. Eur. (Erlanga) 1: 12. 1822.
$=$ Macrosporium Fr., Syst. Mycol. (Lundae) 3: 373. 1832.
$=$ Rhopalidium Mont., Ann. Sci. Nat., Bot., Sér. 2, 6:30. 1836.


Fig. 2. Bayesian 50 \% majority rule consensus tree based on the SSU, LSU and RPB2 sequences of 74 strains representing the Pleosporineae. The Bayesian posterior probabilities (PP) and RAxML bootstrap support values (ML) are given at the nodes (PP/ML). Thickened lines indicate a PP of 1.0 and ML of 100. The tree was rooted to Julella avicenniae (BCC 18422).
= Brachycladium Corda, Icon. Fungorum hucusque Cogn. (Prague) 2: 14. 1838.
= Ulocladium Preuss, Linnaea 24: 111. 1851.
$=$ Chmelia Svob.-Pol., Biologia (Bratislava) 21: 82. 1966.
= Embellisia E.G. Simmons, Mycologia 63: 380. 1971.
$=$ Trichoconiella B.L. Jain, Kavaka 3: 39. 1976 [1975].
= Botryomyces de Hoog \& C. Rubio, Sabouraudia 20: 19. 1982. (nom. illegit.)
$=$ Lewia M.E. Barr \& E.G. Simmons, Mycotaxon 25: 289. 1986.
= Ybotromyces Rulamort, Bull. Soc. Bot. Centre-Ouest, Nouv. Sér. 17: 192. 1986.
$=$ Nimbya E.G. Simmons, Sydowia 41:316. 1989.
$=$ Allewia E.G. Simmons, Mycotaxon 38: 260. 1990.
= Crivellia Shoemaker \& Inderb., Canad. J. Bot. 84: 1308. 2006.
$=$ Chalastospora E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 668. 2007.
= Teretispora E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 674. 2007.
= Undifilum B.M. Pryor, Creamer, Shoemaker, McLain-Romero \& Hambl., Botany 87: 190. 2009.
$=$ Sinomyces Yong Wang bis \& X.G. Zhang, Fungal Biol. 115: 192. 2011.
Colonies effuse, usually grey, dark blackish brown or black. Mycelium immersed or partly superficial; hyphae colourless, olivaceous-brown or brown. Stroma rarely formed. Setae and hyphopodia absent. Conidiophores macronematous, mononematous, simple or irregularly and loosely branched, pale brown or brown, solitary or in fascicles. Conidiogenous cells integrated, terminal becoming intercalary, polytretic, sympodial, or sometimes monotretic, cicatrized. Conidia catenate or solitary, dry, ovoid, obovoid, cylindrical, narrowly ellipsoid or obclavate, beaked or non-beaked, pale or medium olivaceous-brown to brown, smooth or verrucose, with transverse and with or without oblique or longitudinal septa. Septa can be thick, dark and rigid and an internal cell-like structure can be formed. Species with meristematic growth are known.

Ascomata small, solitary to clustered, erumpent to (nearly) superficial at maturity, globose to ovoid, dark brown, smooth, apically papillate, ostiolate. Papilla short, blunt. Peridium thin. Hamathecium of cellular pseudoparaphyses. Asci few to many per ascoma, (4-6-)8-spored, basal, bitunicate, fissitunicate, cylindrical to cylindro-clavate, straight or somewhat curved, with a short, furcate pedicel. Ascospores muriform, ellipsoid to fusoid, slightly constricted at septa, yellow-brown, without guttules, smooth, 3-7 transverse septa, 1-2 series of longitudinal septa through the two original central segments, end cells without septa, or with 1 longitudinal or oblique septum, or with a Y-shaped pair of septa.

Type species: Alternaria alternata (Fr.) Keissl.

## alternaria SECTIONS

Section Alternantherae D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 540. 2013. Fig. 3.

Type species: Alternaria alternantherae Holcomb \& Antonop.
Diagnosis: Section Alternantherae contains short to moderately long conidiophores with a conidiogenous tip which can be enlarged. Conidia are narrowly ellipsoid or ovoid, sometimes


Fig. 3. Alternaria sect. Alternantherae: conidia and conidiophores. A-D. A. alternantherae. E-H. A. perpunctulata. Scale bars $=10 \mu \mathrm{~m}$.
subcylindrical, solitary or rarely paired, sometimes slightly constricted near some septa, longitudinal or oblique septa occasionally occur, disto- and euseptate, with a long apical narrow beak. The conidial beak is unbranched, septate or aseptate, long filiform, and sometimes swollen at the end. Internal compartmentation occurs, cell lumina tend to be broadly octagonal to rounded.

Notes: Section Alternantherae was recently established by Lawrence et al. (2013) after first being described as species-group A. alternantherae (Lawrence et al. 2012). The described section consists of three former Nimbya species which formed a separate clade amidst the Alternaria species-groups based on sequences of the GAPDH, ITS and Alt a 1 genes (Lawrence et al. 2012). Nimbya celosiae is placed in this section based on the data of Lawrence et al. (2012), while $N$. gomphrenae is placed in the section based on ITS sequence data from Chou \& Wu (2002).

Alternaria alternantherae Holcomb \& Antonop., Mycologia 68: 1126. 1976.
$\equiv$ Nimbya alternantherae (Holcomb \& Antonop.) E.G. Simmons \& Alcorn, Mycotaxon 55: 142. 1995.
Alternaria celosiicola Jun. Nishikawa \& C. Nakash., J. Phytopathol. 161: 606. 2013.
Basionym: Nimbya celosiae E.G. Simmons \& Holcomb, Mycotaxon 55: 144. 1995.
$\equiv$ Alternaria celosiae (E.G. Simmons \& Holcomb) D.P. Lawr., M.S. Park \& B.M. Pryor, Mycol. Progr. 11: 811. 2012. (nom. illegit., homonym of Alternaria celosiae (Tassi) O. Savul. 1950).
Alternaria gomphrenae Togashi, Bull. Imp. Coll. Agric. 9: 6. 1926.
$\equiv$ Nimbya gomphrenae (Togashi) E.G. Simmons, Sydowia 41: 324. 1989.

Alternaria perpunctulata (E.G. Simmons) D.P. Lawr., M.S. Park \& B.M. Pryor, Mycol. Progr. 11: 811. 2012.
Basionym: Nimbya perpunctulata E.G. Simmons, Stud. Mycol. 50: 115. 2004.

Section Alternaria D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 538. 2013. Fig. 4.

Type species: Alternaria alternata (Fr.) Keissl.
Diagnosis: Section Alternaria contains straight or curved primary conidiophores, short to long, simple or branched, with one or several apical conidiogenous loci. Conidia are obclavate, long ellipsoid, small or moderate in size, septate, slightly constricted near some septa, with few longitudinal septa, in moderately long to long, simple or branched chains. The conidium body can narrow gradually into a tapered beak or secondary conidiophore. Secondary conidiophores can be formed apically or laterally with one or a few conidiogenous loci.

Notes: Next to the species that are displayed in our phylogeny, 14 more are included in sect. Alternaria based on the study of Lawrence et al. (2013) and confirmed by our molecular data (not shown). We chose not to include 11 species from the study of Lawrence et al. (2013). The species $A$. gossypina, A. grisae, A. grossulariae, A. iridis, A. lini, A. maritima and A. nelumbii were not recognised by Simmons (2007) and the strains of A. malvae, A. rhadina, A. resedae and A. tomato used by Lawrence et al. (2013) were not authentic. Section Alternaria comprises almost 60 Alternaria species based on ITS sequence data (data not shown). The molecular variation within this section is low.

Alternaria alternata (Fr.) Keissl., Beih. Bot. Centralbl., Abt. 2, 29: 434. 1912.
Basionym: Torula alternata Fr., Syst. Mycol. (Lundae) 3: 500. 1832 (nom. sanct.).
= Alternaria tenuis Nees, Syst. Pilze (Würzburg): 72. 1816 [1816-1817].
Additional synonyms listed in Simmons (2007)
Alternaria angustiovoidea E.G. Simmons, Mycotaxon 25: 198. 1986.
Alternaria arborescens E.G. Simmons, Mycotaxon 70: 356. 1999.
Alternaria burnsii Uppal, Patel \& Kamat, Indian J. Agric. Sci. 8: 49. 1938.
Alternaria cerealis E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 600. 2007.
Alternaria citriarbusti E.G. Simmons, Mycotaxon 70: 287. 1999.
Alternaria citrimacularis E.G. Simmons, Mycotaxon 70: 277. 1999.
Alternaria colombiana E.G. Simmons, Mycotaxon 70: 298. 1999.
Alternaria daucifollii E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 518. 2007.
Alternaria destruens E.G. Simmons, Mycotaxon 68: 419. 1998.
Alternaria dumosa E.G. Simmons, Mycotaxon 70: 310. 1999.
Alternaria gaisen Nagano ex Hara, Sakumotsu Byorigaku, Edn 4: 263. 1928.
= Alternaria gaisen Nagano, J. Jap. Soc. Hort. Sci. 32: 16-19. 1920. (nom. illegit.)
= Alternaria kikuchiana S. Tanaka, Mem. Coll. Agric. Kyoto Univ., Phytopathol. Ser. 28: 27. 1933.
= Macrosporium nashi Miura, Flora of Manchuria and East Mongolia, Part III Cryptogams, Fungi: 513. 1928.
Alternaria herbiphorbicola E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 608. 2007.
Alternaria limoniasperae E.G. Simmons, Mycotaxon 70: 272. 1999.


Fig. 4. Alternaria sect. Alternaria: conidia and conidiophores. A, N. A. daucifolii. B, L-M. A. arborescens. C, H-J. A. alternata. D, O. A. gaisen. E. A. limoniasperae. F, K. A. tenuissima. G, P. A. longipes. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria longipes (Ellis \& Everh.) E.W. Mason, Mycol. Pap. 2: 19. 1928.
Basionym: Macrosporium longipes Ellis \& Everh., J. Mycol. 7: 134. 1892.
= Alternaria brassicae var. tabaci Preissecker, Fachliche Mitt. Österr. Tabakregie 16: 4. 1916.

Alternaria perangusta E.G. Simmons, Mycotaxon 70: 303. 1999.
Alternaria postmessia E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 598. 2007.
Alternaria tangelonis E.G. Simmons, Mycotaxon 70: 282. 1999.
Alternaria tenuissima (Nees \& T. Nees : Fr.) Wiltshire, Trans. Brit. Mycol. Soc. 18: 157. 1933.
Basionym: Macrosporium tenuissimum (Nees \& T. Nees) Fr., Syst. Mycol. (Lundae) 3: 374. 1832 (nom. sanct.).
$\equiv$ Helminthosporium tenuissimum Kunze ex Nees \& T. Nees, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 9: 242. 1818.
Additional synonyms listed in Simmons (2007).
Alternaria toxicogenica E.G. Simmons, Mycotaxon 70: 294. 1999.
Alternaria turkisafria E.G. Simmons, Mycotaxon 70: 290. 1999.

Section Brassicicola D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 541. 2013. Fig. 5.

Type species: Alternaria brassicicola (Schwein.) Wiltshire.
Diagnosis: Section Brassicicola contains short to moderately long, simple or branched primary conidiophores with one or several apical conidiogenous loci. Conidia are ellipsoid, ovoid or somewhat obclavate, small or moderate in size, septate, slightly or strongly constricted at most of their transverse septa, with no to many longitudinal septa, in moderately long to long, simple or branched chains, with dark septa and cell walls. Secondary conidiophores can be formed apically or laterally with one or a few conidiogenous loci. Chlamydospores may occur.

Notes: Our molecular data support the morphological placement of $A$. septorioides and $A$. solidaccana in section Brassicicola (Simmons 2007). The other three species were already assigned to this section based on previous molecular studies (Pryor et al. 2009, Runa et al. 2009, Lawrence et al. 2012). Alternaria japonica was previously linked to the A. brassicicola species-group (Pryor \& Gilbertson 2000, Pryor \& Bigelow 2003, Lawrence et al. 2013), but this association was questioned by Hong et al. (2005a). In our analyses, A. japonica clustered in sect. Japonicae.

Alternaria brassicicola (Schwein.) Wiltshire, Mycol. Pap. 20: 8. 1947.
Basionym: Helminthosporium brassicicola Schwein., Trans. Amer. Philos. Soc., Ser. 2, 4: 279. 1832.

Additional synonyms listed in Simmons (2007)
Alternaria conoidea (E.G. Simmons) D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 542. 2013.
Basionym: Embellisia conoidea E.G. Simmons, Mycotaxon 17: 226. 1983.
Alternaria mimicula E.G. Simmons, Mycotaxon 55: 129. 1995.
Alternaria septorioides (Westend.) E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 570. 2007.

Basionym: Sporidesmium septorioides Westend., Bull. Acad. Roy. Sci. Belgique., Cl. Sci., Sér. 2, 21: 236. 1854.
$=$ Alternaria resedae Neerg., Annual Rep. Phytopathol. Lab. J.E. Ohlsens Enkes, Seed Growers, Copenhagen 7: 9.1942 (nom. nud.).


Fig. 5. Alternaria sect. Brassicicola: conidia and conidiophores. A, H. A. brassicicola. B, I, L-M. A. mimicola. C, G. A. solidaccana. D, J-K. A. conoidea. E-F. A. septorioides. Scale bars $=10 \mu \mathrm{~m}$.
$=$ Alternaria resedae Neerg., Danish species of Alternaria \& Stemphylium: 150. 1945. Alternaria solidaccana E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 572. 2007.

Section Chalastospora (E.G. Simmons) Woudenb. \& Crous, comb. et stat. nov. MycoBank MB803733. Fig. 6.
Basionym: Chalastospora E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 668. 2007.

Type species: Alternaria cetera E.G. Simmons.
Diagnosis: Section Chalastospora contains short to long, simple or branched primary conidiophores with one or several conidiogenous loci. Conidia are pale to medium brown, narrowly ellipsoid to ellipsoid or ovoid, beakless, with no to multiple transverse eusepta and


Fig. 6. Alternaria sect. Chalastospora: conidia and conidiophores. A. A. cetera. B. A. obclavata. C. A. breviramosa. D, H. A. armoraciae. E-G. A. abundans. Scale bars $=10 \mu \mathrm{~m}$.
rarely longitudinal septa, solitary or in chains. Secondary conidiophores can be formed apically or laterally with one or a few conidiogenous loci.

Notes: Previous studies already placed E. abundans in the Chalastospora-clade (Andersen et al. 2009, Lawrence et al. 2012). Our study also placed Alternaria armoraciae in this section, while Crous et al. (2009a) showed that Chalastospora gossypii, formerly Alternaria malorum, belonged to this section based on sequences of the ITS and LSU genes.

Alternaria abundans (E.G. Simmons) Woudenb. \& Crous, comb. nov. MycoBank MB803688. Basionym: Embellisia abundans E.G. Simmons, Mycotaxon 17: 222. 1983.
Alternaria armoraciae E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 660. 2007.

Alternaria breviramosa Woudenb. \& Crous, nom. nov. MycoBank MB803690.
Basionym: Chalastospora ellipsoidea Crous \& U. Braun, Persoonia 22: 145. 2009, non Alternaria ellipsoidea E.G. Simmons, 2002.
Etymology: Name refers to the short lateral branches.
Alternaria cetera E.G. Simmons, Mycotaxon 57: 393. 1996.
$\equiv$ Chalastospora cetera (E.G. Simmons) E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 668. 2007.
Alternaria malorum (Ruehle) U. Braun, Crous \& Dugan, Mycol. Progr. 2: 5. 2003.
Basionym: Cladosporium malorum Ruehle, Phytopathology 21: 1146. 1931.
= Cladosporium gossypii Jacz., Khlopkovoe Delo, 1929 (5-6): 564. 1929, non Alternaria gossypii (Jacz.) Y. Nisik., K. Kimura \& Miyaw., 1940.
$\equiv$ Chalastospora gossypii (Jacz.) U. Braun \& Crous, Persoonia 22: 144. 2009.
$=$ Cladosporium malorum Heald, Wash. State Agric. Exp. Sta. Bull., Special Ser. 245: 48. 1930. (nom. nud.)

Additional synonyms in Crous et al. (2009c).


Fig. 7. Alternaria sect. Cheiranthus: conidia and conidiophores. A-B. A. indefessa. B-C. A. cheiranthi. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria obclavata (Crous \& U. Braun) Woudenb. \& Crous, comb. nov. MycoBank MB803689.
Basionym: Chalastospora obclavata Crous \& U. Braun, Persoonia 22: 146. 2009.

Section Cheiranthus Woudenb. \& Crous, sect. nov. MycoBank MB803734. Fig. 7.
Type species: Alternaria cheiranthi (Lib.) P.C. Bolle.
Diagnosis: Section Cheiranthus contains short to moderately long, simple or branched primary conidiophores with one or several conidiogenous loci. Conidia are ovoid, broadly ellipsoid with transverse and longitudinal septa, slightly or strongly constricted at the septa, in short to long, simple or branched chains. Secondary conidiophores can be formed apically or laterally with a single conidiogenous locus.

Notes: Next to Alternaria cheiranthi and Embellisia indefessa, sect. Cheiranthus contains a non-sporulating strain formerly known as Alternaria resedae, CBS 115.44. Because Alternaria resedae is synonymised with Alternaria septorioides (Simmons 2007), which clusters in section Brassisicola, CBS 115.44 will be treated as "Alternaria sp.". Alternaria cheiranthi and E. indefessa have been linked to Ulocladium (Pryor \& Gilbertson 2000, Pryor \& Bigelow 2003, Hong et al. 2005a, Pryor et al. 2009, Runa et al. 2009, Lawrence et al. 2012), but based on morphology could not be placed here. Our extensive dataset showed that they form a sister section to section Ulocladioides.

Alternaria cheiranthi (Lib.) P.C. Bolle, Meded. Phytopathol. Lab. "Willie Commelin Scholten" 7: 43. 1924.
Basionym: Helminthosporium cheiranthi Lib. [as "Helmisporium"], in Desmazières, Plantes Cryptogames du Nord de la France, edn 1: 213. 1827.
$\equiv$ Macrosporium cheiranthi (Lib.) Fr., Syst. Mycol. (Lundae) 3: 374. 1832.
Alternaria indefessa (E.G. Simmons) Woudenberg \& Crous, comb. nov. MycoBank MB803691.
Basionym: Embellisia indefessa E.G. Simmons, Mycotaxon 17: 228. 1983.


Fig. 8. Alternaria sect. Crivellia: conidia and conidiophores. A-B. A. papavericola. C-D. A. penicillata. Scale bars $=10 \mu \mathrm{~m}$.

Section Crivellia (Shoemaker \& Inderb.) Woudenb. \& Crous, comb. et stat. nov. MycoBank MB803735. Fig. 8.
Basionym: Crivellia Shoemaker \& Inderb., Canad. J. Bot. 84: 1308. 2006.
Type species: Alternaria penicillata (Corda) Woudenb. \& Crous (= Cucurbitaria papaveracea De Not.).

Diagnosis: Section Crivellia is characterised by straight or curved, simple or branched primary conidiophores, with geniculate, sympodial proliferations. Conidia are cylindrical, straight to curved to inequilateral, with transverse eusepta, rarely constricted at septa, single or in short, simple or branched chains. Secondary conidiophores are formed apically or laterally. Microsclerotia or chlamydospores may occur. Sexual morphs observed.

Notes: Section Crivellia contains the type species of the sexual morph Crivellia, C. papaveracea, with Brachycladium penicillatum asexual morph, and Brachycladium papaveris. The genus was established by Inderbitzin et al. (2006) based on the finding that C. papaveraceae, formerly Pleospora papaveraceae, belonged to the Alternaria-complex instead of Pleospora s. str. based on ITS, GAPDH and TEF1 sequences.

Alternaria papavericola Woudenb. \& Crous, nom. nov. MycoBank MB803749.
Basionym: Helminthosporium papaveris Sawada, J. Nat. Hist. Soc. Formosa 31: 1. 1917.
$\equiv$ Dendryphion papaveris (Sawada) Sawada, Special Publ. Coll. Agric. Natl. Taiwan Univ. 8: 200. 1959, non Alternaria papaveris (Bres.) M.B. Ellis, 1976.
$\equiv$ Brachycladium papaveris (Sawada) Shoemaker \& Inderb., Canad. J. Bot. 84: 1310. 2006.

Etymology: Name refers to the host.
Alternaria penicillata (Corda) Woudenb. \& Crous, comb. nov. MycoBank MB803692.
Basionym: Brachycladium penicillatum Corda, Icon. Fungorum hucusque Cogn. (Prague) 2: 14. 1838.
$\equiv$ Dendryphion penicillatum (Corda) Fr., Summa Veg. Scand., Sect. Post. (Stockholm): 504. 1849.
$=$ Cucurbitaria papaveracea De Not., Sferiacei Italici: 62. 1863.


Fig. 9. Alternaria sect. Dianthicola: conidia and conidiophores. A-B. A. dianthicola. C-E. A. simsimi. F-H. A. elegans. Scale bars $=10 \mu \mathrm{~m}$.
$\equiv$ Pleospora papaveracea (De Not.) Sacc., Syll. Fungorum (Abellini) 2: 243. 1883.
$\equiv$ Crivellia papaveracea (De Not.) Shoemaker \& Inderb., Canad. J. Bot. 84: 1308. 2006.

Note: The asexual name, Brachycladium penicillatum is older than the sexual name, Cucurbitaria papaveracea, and therefore the species epithet penicillatum is chosen above papaveracea.

Section Dianthicola Woudenb. \& Crous, sect. nov. MycoBank MB803736. Fig. 9.

## Type species: Alternaria dianthicola Neerg.

Diagnosis: Section Dianthicola contains simple or branched primary conidiophores, with or without apical geniculate proliferations. Conidia are narrowly ovoid or narrowly ellipsoid with transverse and few longitudinal septa, slightly constricted at the septa, with a long (filamentous) beak or apical secondary conidiophore, solitary or in short chains.

Note: Based on the ITS sequence, Alternaria dianthicola clustered near Ulocladium (Chou \& Wu 2002). Our extensive dataset places it in a sister section to section Ulocladioides.

Alternaria dianthicola Neerg., Danish species of Alternaria \& Stemphylium: 190. 1945.
Alternaria elegans E.G. Simmons \& J.C. David, Mycotaxon 75: 89. 2000.
Alternaria simsimi E.G. Simmons, Stud. Mycol. 50: 111. 2004.


Fig. 10. Alternaria sect. Embellisia: conidia and conidiophores. A-D. A. embellisia. E-H. A. tellustris. Scale bars $=10 \mu \mathrm{~m}$.

Section Embellisia (E.G. Simmons) Woudenb. \& Crous, comb. et stat. nov. MycoBank MB803737. Fig. 10.
Basionym: Embellisia E.G. Simmons, Mycologia 63: 380. 1971.
Type species: Alternaria embellisia Woudenb. \& Crous ( $\equiv$ Helminthosporium allii Campan., Embellisia allii (Campan.) E.G. Simmons).

Diagnosis: Section Embellisia contains simple, septate conidiophores, straight or with geniculate sympodial proliferation. Condia are solitary, ovoid to subcylindrical, straight to inequilateral, transseptate; septa can be thick, dark and rigid in contrast to the external wall. Chlamydospores may occur.

Notes: Section Embellisia contains the first two species described in the genus Embellisia, Embellisia allii (type species) and Embellisia chlamydospora (Simmons 1971) together with Embellisia tellustris. This clade is also resolved in the latest molecular revision of Embellisia based on sequences of the GAPDH, ITS and Alt a 1 genes as Embellisia group I (Lawrence et al. 2012).

Alternaria chlamydosporigena Woudenb. \& Crous, nom. nov. MycoBank MB803694.
Basionym: Pseudostemphylium chlamydosporum Hoes, G.W. Bruehl \& C.G. Shaw, Mycologia 57: 904. 1965, non Alternaria chlamydospora Mouch., 1973.
$\equiv$ Embellisia chlamydospora (Hoes, G.W. Bruehl \& C.G. Shaw) E.G. Simmons,

Mycologia 63: 384. 1971.
Etymology: Name refers to the formation of chlamydospores during growth.
Alternaria embellisia Woudenb. \& Crous, nom. nov. MycoBank MB803693.
Basionym: Helminthosporium allii Campan., Nuovi Ann. Agric. Roma 4: 87. 1924, non Alternaria allii Nolla, 1927.
$\equiv$ Embellisia allii (Campan.) E.G. Simmons, Mycologia 63: 382. 1971.
Etymology: Name refers to the genus Embellisia for which it served as type species.
Alternaria tellustris (E.G. Simmons) Woudenb. \& Crous, comb. nov. MycoBank MB803695.
Basionym: Embellisia tellustris E.G. Simmons [as "telluster"], Mycotaxon 17: 234. 1983.

Section Embellisioides Woudenb. \& Crous, sect. nov. MycoBank MB803738. Fig. 11.
Type species: Alternaria hyacinthi (de Hoog \& P.J. Mull. bis) Woudenb. \& Crous.
Diagnosis: Section Embellisioides contains simple, septate conidiophores, straight or with multiple, geniculate, sympodial proliferations. Apical or lateral, short secondary conidiophores may occur. Condia are solitary or in short chains, obovoid to ellipsoid, with transverse and longitudinal septa; transverse septa can be thick, dark and rigid in contrast to the external wall. Chlamydospores and a sexual morph may occur.

Note: In Lawrence et al. (2012) the section is named Embellisia group III.
Alternaria botryospora Woudenb. \& Crous, nom. nov. MycoBank MB803705.
Basionym: Embellisia novae-zelandiae E.G. Simmons \& C.F. Hill, Mycotaxon 38: 252. 1990, non Alternaria novae-zelandiae E.G. Simmons, 2002.
Etymology: Name refers to the clusters of conidia.
Alternaria hyacinthi (de Hoog \& P.J. Mull. bis) Woudenb. \& Crous, comb. nov. MycoBank MB803703.
Basionym: Embellisia hyacinthi de Hoog \& P.J. Mull. bis, Netherlands J. Pl. Pathol. 79: 85. 1973.

Alternaria lolii (E.G. Simmons \& C.F. Hill) Woudenb. \& Crous, comb. nov. MycoBank MB803704.
Basionym: Embellisia lolii E.G. Simmons \& C.F. Hill, Stud. Mycol. 50: 113. 2004.
Alternaria planifunda (E.G. Simmons) Woudenb. \& Crous, comb. nov. MycoBank MB803706. Basionym: Embellisia planifunda E.G. Simmons, Mycotaxon 17: 233. 1983.
Alternaria proteae (E.G. Simmons) Woudenb. \& Crous, comb. nov. MycoBank MB803707. Basionym: Embellisia proteae E.G. Simmons, Mycotaxon 38: 258. 1990.
= Allewia proteae E.G. Simmons, Mycotaxon 38: 262. 1990.
Alternaria tumida (E.G. Simmons) Woudenb. \& Crous, comb. nov. MycoBank MB803708. Basionym: Embellisia tumida E.G. Simmons, Mycotaxon 17: 236. 1983.

Section Eureka Woudenb. \& Crous, sect. nov. MycoBank MB803739. Fig. 12.
Type species: Alternaria eureka E.G. Simmons.


Fig. 11. Alternaria sect. Embellisioides: conidia and conidiophores. A-B. A. hyacinthi. C-E. A. lolii. F-H. A. botryospora. I-K. A. planifunda. L-N. A. proteae. O-P. A. tumida. Scale bars $=10 \mu \mathrm{~m}$.


Fig. 12. Alternaria sect. Eureka: conidia and conidiophores. A-B. A. anigozanthi. C-D. A. cumini. E-F. A. leptinellae. G-H. A. triglochinicola. I-J. A. geniostomatis. K-L. A. eureka. Scale bars $=10 \mu \mathrm{~m}$.

Diagnosis: Section Eureka contains simple, septate conidiophores, straight or with geniculate, sympodial proliferations. Apical or lateral, short secondary conidiophores may occur. Condia are solitary or in short chains, narrowly ellipsoid to cylindrical, with transverse and longitudinal septa, slighty constricted at the septa, with a blunt rounded apex. Chlamydospores and a sexual morph may occur.

Notes: Section Eureka contains four Alternaria species and two former Embellisia species. From the Alternaria species only the ITS sequence of $A$. geniostomatis was previously used in a molecular study (Toth et al. 2011), showing it to cluster separate from the other Alternaria spp. The two Embellisia species were included in the latest molecular-based revision of Embellisia (Lawrence et al. 2012) where they formed Embellisia group IV. A sexual morph is known for the type species of this section.

Alternaria anigozanthi Priest, Australas. Pl. Pathol. 24: 239. 1995.
Alternaria cumini E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 664. 2007.
Alternaria eureka E.G. Simmons, Mycotaxon 25: 306. 1986.
$\equiv$ Embellisia eureka (E.G. Simmons) E.G. Simmons, Mycotaxon 38: 260. 1990.
= Lewia eureka E.G. Simmons, Mycotaxon 25: 304. 1986.
$\equiv$ Allewia eureka (E.G. Simmons) E.G. Simmons, Mycotaxon 38: 264. 1990.
Alternaria geniostomatis E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 412. 2007. Alternaria leptinellae (E.G. Simmons \& C.F. Hill) Woudenb. \& Crous, comb. nov. MycoBank MB803696.
Basionym: Embellisia leptinellae E.G. Simmons \& C.F. Hill, Mycotaxon 38: 254. 1990.
Alternaria triglochinicola Alcorn \& S.M. Francis, Mycotaxon 46: 359. 1993.

Section Gypsophilae D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 541. 2013. Fig. 13

Type species: Alternaria gypsophilae Neerg.
Diagnosis: Section Gypsophilae contains simple, or occasionally branched, primary conidiophores, with one or a few conidiogenous loci. Conidia are ellipsoid to long ovoid, with multiple transverse and longitudinal septa, conspicuously constricted near some transverse septa, solitary or in short chains. Secondary conidiophores are formed apically with one or two conidiogenous loci or laterally with a single conidiogenous locus. Species from this section occur on Caryophyllaceae.

Notes: Section Gypsophilae was recently established by Lawrence et al. (2013) containing the four Alternaria species, A. gypsophilae, A. nobilis, A. vaccariae and A. vaccariicola. Our dataset adds four Alternaria species, A. axiaeriisporifera, A. ellipsoidea, A. saponariae, and $A$. juxtiseptata to this section. Simmons (2007) noted the similarity of the primary conidia of $A$. ellipsoidea to A. gypsophilae, A. nobilis, A. saponariae and A. vaccariae. This section contains all Alternaria species that occur on Caryophyllaceae (Simmons 2002), except A. dianthicola which resides in sect. Dianthicola.

Alternaria axiaeriisporifera E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 662. 2007.

Alternaria ellipsoidea E.G. Simmons, Mycotaxon 82: 31. 2002.
Alternaria gypsophilae Neerg., Danish species of Alternaria \& Stemphylium: 207. 1945.
Alternaria juxtiseptata E.G. Simmons, Mycotaxon 82: 32. 2002.
Alternaria nobilis (Vize) E.G. Simmons, Mycotaxon 82: 7. 2002.
Basionym: Macrosporium nobile Vize, Grevillea 5(35): 119. 1877.
Alternaria saponariae (Peck) Neerg., Annual Rep. Phytopathol. Lab. J.E. Ohlsens Enkes, Seed Growers, Copenhagen 3: 6. 1938 [1937-1938].
Basionym: Macrosporium saponariae Peck, Rep. (Annual) NewYork State Mus. Nat. Hist. 28: 62. 1876 [1875].

Alternaria vaccariae (Săvul. \& Sandu) E.G. Simmons \& S.T. Koike, Mycotaxon 82: 21. 2002. Basionym: Macrosporium vaccariae Săvul. \& Sandu, Hedwigia 73: 130. 1933.
Alternaria vaccariicola E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 594. 2007.


Fig. 13. Alternaria sect. Gypsophilae: conidia and conidiophores. A-B. A. axiariisporifera. C-D. A. ellipsoidea. E-G. A. saponariae. H-I. A. vaccariae. J-K. A. nobilis. L-M. A. juxtiseptata. N-P. A. vaccariicola. Scale bars $=10 \mu \mathrm{~m}$.


Fig. 14. Alternaria sect. Infectoriae: conidia and conidiophores. A-B. A. ethzedia. C-D. A. infectoria. E-F. A. conjuncta. G-H. A. oregonensis. Scale bars $=10 \mu \mathrm{~m}$.

Section Infectoriae Woudenb. \& Crous, sect. nov. MycoBank MB803740. Fig. 14.
Type species: Alternaria infectoria E.G. Simmons.
Diagnosis: Section Infectoriae contains short to long, simple or branched primary conidiophores with one or several conidiogenous loci. Conidia are obclavate, long-ellipsoid, small or moderate in size, septate, slightly constricted near some septa, with few longitudinal septa, in moderately long to long, branched chains. Long, geniculate, multi-locus secondary conidiophores can be formed apically or laterally. Sexual morphs are known, and meristematic growth has been reported.

Notes: In addition to the six species that are displayed in our phylogeny, 19 more are included based on the study of Lawrence et al. (2013), confirmed with our molecular data (not shown). From these 25 species, nine species have a known sexual morph in Lewia. Three species from the study of Lawrence et al. (2013) are not included; A. photistica (sect. Panax) and A. dianthicola (sect. Dianthicola) cluster elsewhere in our phylogenies and $A$. peglionii is marked as a taxon incertae sedis by Simmons (2007). The human pathogenic genera Ybotromyces and Chmelia are also embedded in sect. Infectoriae.

Alternaria alternarina E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 644. 2007.
= Pyrenophora alternarina M.D. Whitehead \& J. Dicks., Mycologia 44: 748. 1952.
$\equiv$ Lewia alternarina (M.D. Whitehead \& J.G. Dicks.) E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 644. 2007.

Alternaria arbusti E.G. Simmons, Mycotaxon 48: 103. 1993.
Alternaria caespitosa (de Hoog \& C. Rubio) Woudenb. \& Crous, comb. nov. MycoBank MB803698.
Basionym: Botryomyces caespitosus de Hoog \& C. Rubio, Mycotaxon 14: 19. 1982. $\equiv$ Ybotromyces caespitosus (de Hoog \& C. Rubio) Rulamort, Bull. Soc. Bot. CentreOuest, Nouv. Sér. 21: 512. 1990.
Alternaria californica E.G. Simmons \& S.T. Koike, CBS Biodiversity Ser. (Utrecht) 6: 602. 2007.

Alternaria conjuncta E.G. Simmons, Mycotaxon 25: 294. 1986.
$=$ Sphaeria scrophulariae Desm., Ann. Sci. Nat., Bot., Sér. 2, 6: 245. 1836.
$\equiv$ Leptosphaeria scrophulariae (Desm.) Sacc., Syll. Fungorum (Abellini) 2: 57. 1883.
$\equiv$ Heptameria scrophulariae (Desm.) Cooke, Grevillea 18(no. 86): 31. 1889.
$\equiv$ Pleospora scrophulariae (Desm.) Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.Naturwiss. Cl., Abt. 1. 126(4-5): 374. 1917.
$\equiv$ Lewia scrophulariae (Desm.) M.E. Barr \& E.G. Simmons, Mycotaxon 25: 294. 1986.
Alternaria daucicaulis E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 640. 2007.
= Lewia daucicaulis E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 640. 2007.
Alternaria ethzedia E.G. Simmons, Mycotaxon 25: 300. 1986.
$=$ Lewia ethzedia E.G. Simmons, Mycotaxon 25: 299. 1986.
Alternaria frumenti E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 620. 2007. Alternaria graminicola E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 626. 2007.
Alternaria hordeiaustralica E.G. Simmons \& Alcorn, CBS Biodiversity Ser. (Utrecht) 6: 614. 2007.
= Lewia hordeiaustralica E.G. Simmons \& Alcorn, CBS Biodiversity Ser. (Utrecht) 6: 614. 2007.

Alternaria hordeicola E.G. Simmons \& Kosiak, CBS Biodiversity Ser. (Utrecht) 6: 630. 2007.
= Lewia hordeicola Kwaśna \& Kosiak, Mycologia 98: 663. 2006.
Alternaria humuli E.G. Simmons, Mycotaxon 83: 139. 2002.
Alternaria incomplexa E.G. Simmons, Mycotaxon 57: 394. 1996.
Alternaria infectoria E.G. Simmons, Mycotaxon 25: 298. 1986.
= Pleospora infectoria Fuckel, Jahrb. Nassauischen Vereins Naturk. 23-24: 132. 1870 [1869-70].
$\equiv$ Sphaeria infectoria (Fuckel) Cooke, Handb. Brit. Fungi 2: 897. 1871.
$\equiv$ Pleospora phaeocomoides var. infectoria (Fuckel) Wehm., A World Monograph of the Genus Pleospora and its Segregates: 121. 1961.
$\equiv$ Lewia infectoria (Fuckel) M.E. Barr \& E.G. Simmons, Mycotaxon 25: 296. 1986.
Alternaria intercepta E.G. Simmons, Mycotaxon 83: 134. 2002.
$=$ Lewia intercepta E.G. Simmons \& McKemy, Mycotaxon 83: 133. 2002.
Alternaria merytae E.G. Simmons, Mycotaxon 83: 136. 2002.
Alternaria metachromatica E.G. Simmons, Mycotaxon 50: 418. 1994.
Alternaria novae-zelandiae E.G. Simmons, Mycotaxon 83: 142. 2002.
Alternaria oregonensis E.G. Simmons, Mycotaxon 50: 417. 1994.
Alternaria slovaca (Svob.-Pol., L. Chmel \& Bojan.) Woudenb. \& Crous, comb. nov. MycoBank MB803699.
Basionym: Aureobasidium slovacum Svob.-Pol., L. Chmel \& Bojan., Conspect. Verruc. 5: 116. 1966. $\equiv$ Chmelia slovaca (Svob.-Pol., L. Chmel \& Bojan.) Svob.-Pol., Biologia (Bratislava) 21: 83. 1966.

Alternaria triticimaculans E.G. Simmons \& Perelló, Mycotaxon 50: 413. 1994.
Alternaria triticina Prasada \& Prabhu, Indian Phytopathol. 15 (3-4): 292. 1963. [1962]
Alternaria ventricosa R.G. Roberts, Mycotaxon 100: 164. 2007.
Alternaria viburni E.G. Simmons, Mycotaxon 83: 132. 2002.
$=$ Lewia viburni E.G. Simmons \& McKemy, Mycotaxon 83: 130. 2002.

Section Japonicae Woudenb. \& Crous, sect. nov. MycoBank MB803741. Fig. 15.
Type species: Alternaria japonica Yoshii.
Diagnosis: Section Japonicae contains short to long, simple or occasionally branched primary conidiophores with a single conidiogenous locus. Conidia are short, to long-ovoid with transverse and longitudinal septa, conspicuously constricted at most of the transverse septa, in short chains. Apical secondary conidiophores are produced with a single conidiogenous locus. The species within this section occur on Brassicaceae.

Note: Alternaria japonica was previously connected to the A. brassicicola species-group (Pryor \& Gilbertson 2000, Pryor \& Bigelow 2003, Lawrence et al. 2013), but this association was questioned by Hong et al. (2005a).

Alternaria japonica Yoshii, J. Pl. Protect. 28: 17. 1941.
= Alternaria matthiolae Neerg., Danish species of Alternaria and Stemphylium: 184. 1945.

Alternaria nepalensis E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 480. 2007.

Section Nimbya (E.G. Simmons) Woudenb. \& Crous, comb. et stat. nov. MycoBank MB803742. Fig. 16.
Basionym: Nimbya E.G. Simmons, Sydowia 41: 316. 1989.
Type species: Alternaria scirpicola (Fuckel) Sivan.
Diagnosis: Section Nimbya contains simple, short to moderately long conidiophores, which may form one or a few short to long, geniculate, sympodial proliferations. Conidia are narrowly elongate-obclavate, gradually tapering apically, solitary or in short chains, with transverse disto- and eusepta, sometimes slightly constricted near eusepta. Apical condiophores with a single conidiogenous locus can be formed. Internal compartmentation occurs, cell lumina tend to be broadly octagonal to rounded. A sexual morph may occur.

Notes: Section Nimbya contains the type species of Nimbya, N. scirpicola, and N. caricis (Simmons 1989). A more extensive study on Nimbya (Lawrence et al. 2012) found that $N$. scirpinfestans and N. scirpivora also belonged to this section based on sequences of the GAPDH, ITS and Alt a 1 genes.

Alternaria caricis (E.G. Simmons) Woudenb. \& Crous, comb. nov. MycoBank MB803700. Basionym: Nimbya caricis E.G. Simmons, Sydowia 41: 328. 1989.


Fig. 15. Alternaria sect. Japonicae: conidia and conidiophores. A-B. A. japonica. C-E. A. nepalensis. Scale bars $=10 \mu \mathrm{~m}$.


Fig. 16. Alternaria sect. Nimbya: conidia and conidiophores. A-B. A. caricis. C-D. A. scirpicola. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria scirpicola (Fuckel) Sivan., Bitunicate Ascomycetes and their Anamorphs (Vaduz): 526. 1984.

Basionym: Sporidesmium scirpicola Fuckel, Jahrb. Nassauischen Vereins Naturk. 23-24: 140. 1870 [1869-70].
$\equiv$ Clasterosporium scirpicola (Fuckel) Sacc., Syll. Fungorum (Abellini) 4: 393. 1886.
$\equiv$ Cercospora scirpicola (Fuckel) Zind.-Bakker, Rev. Mycol. (Paris) 5: 66. 1940.
$\equiv$ Alternaria scirpicola (Fuckel) M.T. Lucas \& J. Webster, Čas. Slez. Mus., Ser. A, Hist. Nat. 23: 151. 1974 (nom. inval.).
$\equiv$ Nimbya scirpicola (Fuckel) E.G. Simmons, Sydowia 41:316. 1989.
$=$ Sphaeria scirpicola DC., in Lamarck \& de Candolle, Fl. Franç., Edn 3 (Paris) 2: 300. 1805.
$\equiv$ Clathrospora scirpicola (DC.) Höhn., Ann. Mycol. 18(1/3): 77. 1920.
$\equiv$ Macrospora scirpicola (DC.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23-24: 139. 1870 [1869-70].

三 Pyrenophora scirpicola (DC.) E. Müll., Sydowia 5(3-6): 256. 1951.
Note: Although Sphaeria scirpicola DC. (de Candolle 1805) predates Sporidesmium scirpicola Fuckel (Fuckel 1870), a valid combination in Alternaria already exists, thus we chose to


Fig. 17. Alternaria sect. Panax: conidia and conidiophores. A-B. A. avenicola. C-D. A. calycipyricola. E-F. A. panax. G-H. A. photistica. Scale bars $=10 \mu \mathrm{~m}$.
retain Alternaria scirpicola (Fuckel) Sivan., which is also a well established name.
Alternaria scirpinfestans (E.G. Simmons \& D.A. Johnson) Woudenb. \& Crous, comb. nov. MycoBank MB803701.
Basionym: Nimbya scirpinfestans E.G. Simmons \& D.A. Johnson, Mycotaxon 84: 420. 2002. $=$ Macrospora scirpinfestans E.G. Simmons \& D.A. Johnson, Mycotaxon 84: 417. 2002. Alternaria scirpivora (E.G. Simmons \& D.A. Johnson), Woudenb. \& Crous, comb. nov. MycoBank MB803702.
Basionym: Nimbya scirpivora E.G. Simmons \& D.A. Johnson, Mycotaxon 84: 424. 2002. $=$ Macrospora scirpivora E.G. Simmons \& D.A. Johnson, Mycotaxon 84: 422. 2002.

Section Panax D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 541. 2013. Fig. 17.
Type species: Alternaria panax Whetzel.
Diagnosis: Section Panax contains simple or branched, short to moderately long primary conidiophores, with one or a few conidiogenous loci. Conidia are obclavate to ovoid, with multiple transverse and longitudinal septa, conspicuously constricted near several transverse septa, solitary or in simple or branched, short chains. Apical secondary conidiophores are formed with one or several conidiogenous loci, multiple lateral secondary conidiophores with a single conidiogenous locus may occur.

Notes: Section Panax was recently described by Lawrence et al. (2013) and consists of $A$. calycipyricola, A. eryngii and A. panax. Our extended dataset added the species A. avenicola and $A$. photistica to this section. Three species, A. avenicola, A. calycipyricola, and A. photistica have earlier been placed in the $A$. infectoria species-group based on their morphological characters (Simmons 2007), and two of them have a known sexual morph; Lewia avenicola (Simmons 2007) and Lewia photistica (Simmons 1986). A phylogenetic study based on Alt a 1 and GAPDH sequences placed $A$. photistica in the $A$. infectoria species-group (Hong et al. 2005) but an extensive study on the $A$. infectoria species-group (Andersen et al. 2009) confirmed our finding, and placed this species outside the $A$. infectoria species-group. Additional research performed on multiple $A$. photistica strains support our sequence data (data not shown).

Alternaria avenicola E.G. Simmons, Kosiak \& Kwaśna, in Simmons, CBS Biodiversity Ser. (Utrecht) 6: 114. 2007.
$=$ Lewia avenicola Kosiak \& Kwaśna, Mycol. Res. 107: 371. 2003.
Alternaria calycipyricola R.G. Roberts, Mycotaxon 100: 162. 2007.
Alternaria eryngii (Pers.) S. Hughes \& E.G. Simmons, Canad. J. Bot. 36: 735. 1958.
Basionym: Conoplea eryngii Pers., Mycol. Eur. (Erlanga) 1: 11. 1822.
$\equiv$ Exosporium eryngianum (Pers.) Chevall., Flore Générale des Environs de Paris 1: 39. 1826.
$\equiv$ Exosporium eryngii (Pers.) Duby, Bot. Gallicum., Edn 2 (Paris) 2: 882. 1830.
$\equiv$ Helminthosporium eryngii (Pers.) Fr., Syst. Mycol. (Lundae) 3: 361. 1832.
Alternaria panax Whetzel, Bull. U.S.D.A. 250: 11. 1912.
= Macrosporium araliae Dearn. \& House, Circ. New York State Mus. 24: 58. 1940.
$=$ Alternaria araliae H.C. Greene, Trans. Wisconsin Acad. Sci. 42: 80. 1953.
Alternaria photistica E.G. Simmons, Mycotaxon 25: 304. 1986.
$=$ Lewia photistica E.G. Simmons, Mycotaxon 25: 302. 1986.

Section Phragmosporae Woudenb. \& Crous, sect. nov. MycoBank MB803743. Fig. 18.
Type species: Alternaria phragmospora Emden.
Diagnosis: Section Phragmosporae contains simple, short to moderately long, primary conidiophores, with one or multiple geniculate, sympodial proliferations. Conidia are (broad) ovoid to long ovoid, ellipsoid, curved, or limaciform, with multiple transverse and few to multiple longitudinal septa, some septa darkened, slightly to conspicuously constricted near several transverse septa, solitary or in simple short chains. Apical secondary conidiophores are formed with one or several conidiogenous loci. All species within the section are known from soil and seawater environments.

Note: Section Phragmosporae contains six species of which two were linked to Embellisia.
Alternaria chlamydospora Mouch. [as "chlamydosporum"], Mycopathol. Mycol. Appl. 50: 217. 1973.

Alternaria didymospora (Munt.-Cvetk.) Woudenb. \& Crous, comb. nov. MycoBank MB803709. Basionym: Embellisia didymospora Munt.-Cvetk., Mycologia 68: 49. 1976.
Alternaria limaciformis E.G. Simmons, Mycotaxon 13: 24. 1981.


Fig. 18. Alternaria sect. Phragmosporae: conidia and conidiophores. A-B. A. didymospora. C. A. phragmospora. D-E. A. limaciformis. F-G. A. molesta. H-I. A. mouchaccae. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria molesta E.G. Simmons, Mycotaxon 13: 17. 1981. Alternaria mouchaccae E.G. Simmons, Mycotaxon 13: 18. 1981.

三 Ulocladium chlamydosporum Mouch., Rev. Mycol. (Paris) 36: 114. 1971, non Alternaria chlamydospora Mouch., 1973.
Alternaria phragmospora Emden, Acta Bot. Neerl. 19: 393. 1970.
$\equiv$ Embellisia phragmospora (Emden) E.G. Simmons, Mycotaxon 17: 232. 1983.

Section Porri D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 541. 2013. Fig. 19
Type species: Alternaria porri (Ellis) Cif.
Diagnosis: Section Porri is characterised by broadly ovoid, obclavate, ellipsoid, subcylindrical or obovoid (medium) large conidia, disto- and euseptate, solitary or in short to moderately long chains, with a simple or branched, long to filamentous beak. Conidia contain multiple transverse and longitudinal septa and are slightly constricted near some transverse septa. Secondary conidiophores can be formed apically or laterally.

Notes: In addition to the six species that are displayed in our phylogeny, 40 more are included based on the study of Lawrence et al. (2013), confirmed with own molecular data (not shown). With almost 80 species section Porri is the largest Alternaria section (data


Fig. 19. Alternaria sect. Porri: conidia and conidiophores. A-C. A. daucii. D-F. A. pseudorostrata. G-H. A. solani. Scale bars $=10 \mu \mathrm{~m}$.
not shown). The section displays a higher level of genetic variation than the second largest section; section Alternaria.

Alternaria acalyphicola E.G. Simmons, Mycotaxon 50: 260. 1994.
Alternaria agerati Sawada ex E.G. Simmons, Mycotaxon 65: 63. 1997.
$=$ Alternaria agerati Sawada, Rep. Dept. Agric. Gov. Res. Inst. Formosa 86: 165. 1943. (nom. inval., Art. 36.1)
Alternaria agripestis E.G. Simmons \& K. Mort., Mycotaxon 50: 255. 1994.
Alternaria anagallidis A. Raabe, Hedwigia 78: 87. 1939.
Alternaria aragakii E.G. Simmons, Mycotaxon 46: 181. 1993.
Alternaria argyroxiphii E.G. Simmons \& Aragaki, Mycotaxon 65: 40. 1997.
Alternaria bataticola Ikata ex W. Yamam., Trans. Mycol. Soc. Japan 2(5): 89. 1960.
= Macrosporium bataticola Ikata, Agric. Hort. (Tokyo) 22: 241. 1947 (nom. inval., Art. 36.1).

Alternaria blumeae E.G. Simmons \& Sontirat, Mycotaxon 65: 81. 1997.
Alternaria calendulae Ondřej, Čas. Slez. Mus. v Opavĕ, Ser. A, Hist. Nat. 23(2): 150. 1974.
= Alternaria calendulae W. Yamam. 1939 (nom. nud.).
= Macrosporium calendulae Nelen, Bull. Centr. Bot. Gard. (Moscow) 35: 90. 1959 (nom. inval., Art. 36.1).
$=$ Macrosporium calendulae Nelen, Bot. Mater. Otd. Sporov. Rast. Bot. Inst. Akad. Nauk S.S.S.R. 15: 144. 1962.
= Alternaria calendulae Nirenberg, Phytopathol. Z. 88(2): 108. 1977 (nom. illegit., Art. 53.1).

Alternaria capsici E.G. Simmons, Mycotaxon 75: 84. 2000.
Alternaria carthami S. Chowdhury, J. Indian Bot. Soc. 23: 65. 1944.
$=$ Macrosporium anatolicum A. Săvul., Bull. Sect. Sci. Acad. Roumaine 26: 709. 1944.
Alternaria cassiae Jurair \& A. Khan, Pakistan J. Sci. Industr. Res. 3(1): 72. 1960.
Alternaria cichorii Nattrass, First List of Cyprus Fungi: 29. 1937.
$\equiv$ Alternaria porri f. sp. cichorii (Natrass) T. Schmidt, Pflanzenschutz-berichte 32: 181. 1965.
$\equiv$ Macrosporium cichorii (Nattrass) Gordenko, Mikol. Fitopatol. 9(3): 241. 1975.
Alternaria cirsinoxia E.G. Simmons \& K. Mort., Mycotaxon 65: 72. 1997.
Alternaria crassa (Sacc.) Rands, Phytopathology 7: 337. 1917.
Basionym: Cercospora crassa Sacc., Michelia 1(no. 1): 88. 1877.
Alternaria cretica E.G. Simmons \& Vakal., Mycotaxon 75: 64. 2000.
Alternaria cucumerina (Ellis \& Everh.) J.A. Elliott, Amer. J. Bot. 4: 472. 1917.
Basionym: Macrosporium cucumerinum Ellis \& Everh., Proc. Acad. Nat. Sci. Philadelphia 47: 440. 1895.

Alternaria cyphomandrae E.G. Simmons, Mycotaxon 75: 86. 2000.
Alternaria danida E.G. Simmons, Mycotaxon 65: 78. 1997.
Alternaria dauci (J.G. Kühn) J.W. Groves \& Skolko, Canad. J. Res., Sect. C, Bot. Sci. 22: 222. 1944.

Basionym: Sporidesmium exitiosum var. dauci J.G. Kühn, Hedwigia 1: 91. 1855.
Additional synonyms in Simmons 2007.
Alternaria dichondrae Gambogi, Vannacci \& Triolo, Trans. Brit. Mycol. Soc. 65(2): 323. 1975.
Alternaria euphorbiicola E.G. Simmons \& Engelhard, Mycotaxon 25: 196. 1986.
$\equiv$ Macrosporium euphorbiae Reichert, Bot. Jahrb. Syst. 56: 723. 1921. (nom. illegit., Art 53.1).

Alternaria grandis E.G. Simmons, Mycotaxon 75: 96. 2000.
Alternaria hawaiiensis E.G. Simmons, Mycotaxon 46: 184. 1993.
Alternaria limicola E.G. Simmons \& M.E. Palm, Mycotaxon 37: 82. 1990.
Alternaria linicola J.W. Groves \& Skolko, Canad. J. Res., Sect. C, Bot. Sci. 22: 223. 1944.
Alternaria macrospora Zimm., Ber. Land-Forstw. Deutsch-Ostafrika 2: 24. 1904.
$\equiv$ Macrosporium macrosporum (Zimm.) Nishikado \& Oshima, Agric. Res. (Kurashiki) 36: 391. 1944.
= Sporidesmium longipedicellatum Reichert, Bot. Jahrb. Syst. 56: 723. 1921.
$\equiv$ Alternaria longipedicellata (Reichert) Snowden, Rep. Dept. Agric. Uganda: 31. 1927 [1926].
Alternaria multirostrata E.G. Simmons \& C.R. Jacks., Phytopathology 58: 1139. 1968.
Alternaria nitrimali E.G. Simmons \& M.E. Palm, Mycotaxon 75: 93. 2000.
Alternaria passiflorae J.H. Simmonds, Proc. Roy. Soc. Queensland. 49: 151. 1938.
Alternaria poonensis Ragunath, Mycopathol. Mycol. Appl. 21: 315. 1963.
Alternaria porri (Ellis) Cif., J. Dept. Agric. Porto Rico 14: 30. 1930 [1929].
Basionym: Macrosporium porri Ellis, Grevillea 8 (no. 45): 12. 1879.
Alternaria protenta E.G. Simmons, Mycotaxon 25: 207. 1986.
Alternaria pseudorostrata E.G. Simmons, Mycotaxon 57: 398. 1996.
Alternaria ricini (Yoshii) Hansf., Proc. Linn. Soc. Lond. : 53. 1943.
Basionym: Macrosporium ricini Yoshii, Bult. Sci. Fak. Terk. Kjusu Imp. Univ. 3(4): 327. 1929.

Alternaria rostellata E.G. Simmons, Mycotaxon 57: 401. 1996.
Alternaria scorzonerae (Aderh.) Loer., Netherlands J. Pl. Pathol. 90(1): 37. 1984.
Basionym: Sporidesmium scorzonerae Aderh.,Arbeiten Kaiserl. Biol. Anst. Land-Forstw . 3: 439. 1903.

Alternaria sesami (E. Kawam.) Mohanty \& Behera, Curr. Sci. 27: 493. 1958.
Basionym: Macrosporium sesami E. Kawam., Fungi 1(2): 27. 1931.
Alternaria solani Sorauer, Z. Pflanzenkrankh. Pflanzenschutz 6: 6. 1896.
$=$ Macrosporium solani Ellis \& G. Martin, Amer. Naturalist 16(12): 1003. 1882 $\equiv$ Alternaria solani (Ellis \& G. Martin) L.R. Jones \& Grout, Vermont Agric. Exp. Sta. Annual Rep. 9: 86. 1896.
Additional synonyms in Simmons (2007).
Alternaria solani-nigri R. Dubey, S.K. Singh \& Kamal [as "solani-nigrii"], Microbiol. Res. 154(2): 120. 1999.
Alternaria steviae Ishiba, T. Yokoy. \& Tani, Ann. Phytopathol. Soc. Japan 48(1): 46. 1982.
Alternaria subcylindrica E.G. Simmons \& R.G. Roberts, Mycotaxon 75: 62. 2000.
Alternaria tagetica S.K. Shome \& Mustafee, Curr. Sci. 35: 370. 1966.
Alternaria tomatophila E.G. Simmons, Mycotaxon 75: 53. 2000.
Alternaria tropica E.G. Simmons, Mycotaxon 46: 187. 1993.
Alternaria zinniae H.Pape ex M.B. Ellis, Mycol. Pap. 131: 22. 1972.
= Alternaria zinniae H. Pape, Angew. Bot. 24: 61. 1942. (nom. inval., Art. 36.1)

Section Pseudoulocladium Woudenb. \& Crous, sect. nov. MycoBank MB803744. Fig. 20.
Type species: Alternaria chartarum Preuss.
Diagnosis: Section Pseudoulocladium is characterised by simple or branched conidiophores with short, geniculate, sympodial proliferations. Conidia are obovoid, non-beaked with a narrow base, in simple or (mostly) branched chains. Apical secondary conidiophores with multiple conidiogenous loci and lateral secondary conidiophores with a single conidiogenous locus can be formed.

Note: It forms a sister clade to section Ulocladioides.

Alternaria aspera Woudenb. \& Crous, nom. nov. MycoBank MB803712.
Basionym: Ulocladium arborescens E.G. Simmons, Stud. Mycol. 50: 117. 2004, non Alternaria arborescens E.G. Simmons, 1999.
Etymology: Name refers to the conspicuously ornamented conidia.
Alternaria chartarum Preuss, Bot. Zeitung 6: 412, 1848.
$\equiv$ Sporidesmium polymorphum var. chartarum (Preuss) Cooke, Fungi Brit. Exs., ser. 2: 329. 1875.
$\equiv$ Ulocladium chartarum (Preuss) E.G. Simmons, Mycologia 59: 88. 1967.
= Alternaria stemphylioides Bliss, Mycologia 36: 538. 1944.
$\equiv$ Alternaria chartarum f. stemphylioides (Bliss) P. Joly, Encycl. Mycol. (Paris) 33: 161. 1964.

Alternaria concatenata Woudenb. \& Crous, nom. nov. MycoBank MB803713.
Basionym: Ulocladium capsici F. Xue \& X.G. Zhang [as "capsicuma"], Sydowia 59: 174. 2007, non Alternaria capsici E.G. Simmons, 2000.


Fig. 20. Alternaria sect. Pseudoulocladium: conidia and conidiophores. A-B. A. aspera. C-D. A. concatenata. E-F. A. chartarum. G-H. A. septospora. Scale bars $=10 \mu \mathrm{~m}$.

Etymology: Name refers to the concatenated conidia.
Alternaria septospora (Preuss) Woudenb. \& Crous, comb. nov. MycoBank MB803714.
Basionym: Helminthosporium septosporum Preuss, Linnaea 24: 117. 1851.
$\equiv$ Macrosporium septosporum (Preuss) Rabenh., Bot. Zeitung 9: 454. 1851.
$\equiv$ Ulocladium septosporum (Preuss) E.G. Simmons, Mycologia 59: 87. 1967.

Section Radicina D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 541. 2013. Fig. 21.

Type species: Alternaria radicina Meier, Drechsler \& E.D. Eddy.
Diagnosis: Section Radicina contains straight, simple or branched, short or long, primary conidiophores with multiple, short geniculate, sympodial proliferations with single or a few conidiogenous loci at the apex. Sporulation resembles a cluster or clumps of conidia. Conidia are widely ovoid to narrowly ellipsoid, moderate in size, beakless, with several transverse and longitudinal septa, solitary or in short chains. Solitary, short, apical secondary conidiophores may occur. The species from this section occur on Umbelliferae.

Note: This section was first recognised by Pryor \& Gilbertson (2000) based on sequence data of the ITS and mitochondrial SSU.


Fig. 21. Alternaria sect. Radicina: conidia and conidiophores. A-C.A. carotiincultae. D-E. A. petroselini. F-G. A. radicina. H-I. A. selini. J-L. A. smyrnii. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria carotiincultae E.G. Simmons, Mycotaxon 55: 103. 1995.
Alternaria petroselini (Neerg.) E.G. Simmons, More dematiaceous hyphomycetes (Kew): 417. 1976.

Basionym: Stemphylium petroselini Neerg., Zentralbl. Bakteriol., 2. Abt., 104: 411. 1942.
$\equiv$ Stemphylium radicinum var. petroselini (Neerg.) Neerg., Danish species of Alternaria \& Stemphylium: 357. 1945.
$\equiv$ Alternaria radicina var. petroselini (Neerg.) Neerg., Encycl. Mycol. 33: 123. 1964.
Alternaria radicina Meier, Drechsler \& E.D. Eddy, Phytopathology 12: 157. 1922.
$\equiv$ Stemphylium radicinum (Meier, Drechsler \& E.D. Eddy) Neerg., Annual Rep.
Phytopathol. Lab. J.E. Ohlsens Enkes, Seed Growers, Copenhagen 4: 14. 1939.

[^0]Section Sonchi D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 542. 2013. Fig. 22.

Type species: Alternaria sonchi Davis.
Diagnosis: Section Sonchi is characterised by subcylindrical, broadly ovoid, broadly ellipsoid or obclavate, (medium) large conidia, single or in short chains, with multiple transverse and few longitudinal septa, slightly constricted at the septa, with a blunt taper which can form secondary conidiophores.

Notes: The species-group was described by Hong et al. (2005a) based on molecular data of the GAPDH and Alt a 1 regions. Lawrence et al. (2013) included A. brassicae as a basal lineage in sect. Sonchi, which is supported as a monotypic lineage in our analyses. The species from section Sonchi occur on multiple hosts within the Compositae.

Alternaria cinerariae Hori \& Enjoji, J. Pl. Protect. 18: 432. 1931.
Alternaria sonchi Davis, in Elliott, Bot. Gaz. 62: 416. 1916.

Section Teretispora (E.G. Simmons) Woudenb. \& Crous, comb. et stat. nov. MycoBank MB803745. Fig. 23.
Basionym: Teretispora E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 674. 2007.
Type species: Alternaria leucanthemi Nelen.
Diagnosis: Section Teretispora is characterised by simple conidiophores, sometimes extending at the apex with one or two, geniculate, sympodial proliferations, bearing single, long cylindrical mature conidia lacking a beak portion, with many transverse and a few longitudinal septa, constricted at most of the transverse septa. Secondary conidiophores with a single conidium are rarely formed at the apex; instead, they may form from the base of the primary conidium.

Notes: The genus Teretispora had Teretispora leucanthemi, formerly Alternaria leucanthemi (= Alternaria chrysanthemi), as type and only species (Simmons 2007). We chose to treat this as a section, which retains the name Teretispora, rather than a monotypic lineage.


Fig. 22. Alternaria sect. Sonchi: conidia and conidiophores. A-B. A. cinerariae. C-D. A. sonchi. Scale bars $=10 \mu \mathrm{~m}$.


Fig. 23. Alternaria sect. Teretispora: conidia and conidiophores. A-D. A. leucanthemi. Scale bars $=10$ $\mu \mathrm{m}$.

Alternaria leucanthemi Nelen, in Nelen \& Vasiljeva, Bot. Mater. Otd. Sporov. Rast. Bot. Inst. Akad. Nauk S.S.S.R. 15: 148. 1962.
$\equiv$ Teretispora leucanthemi (Nelen) E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 674. 2007.
= Alternaria leucanthemi Nelen, Bull. Centr. Bot. Gard. (Moscow) 35: 83. 1959. (nom. inval., Art. 36.1)
= Alternaria chrysanthemi E.G. Simmons \& Crosier, Mycologia 57: 142. 1965.

Section Ulocladioides Woudenb. \& Crous, sect. nov. MycoBank MB803746. Fig. 24.
Type species: Alternaria cucurbitae Letendre \& Roum.
Diagnosis: Section Ulocladioides is characterised by conidiophores with short, geniculate, sympodial proliferations. Conidia are obovoid, non-beaked with a narrow base, single or in chains, which may form secondary conidiophores at the apex.

Note: Section Ulocladioides resembles section Ulocladium and contains the majority of the species included in this study from the genus Ulocladium (11/17).


Fig. 24. Alternaria sect. Ulocladioides: conidia and conidiophores. A-B. A. atra. C-D. A. brassicaepekinensis. E-F. A. cantlous. G-H. A. multiformis. I-J. A. obovoidea. K-L. A. heterospora. M-N. A. subcucurbitae. O-P. A. terricola. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria atra (Preuss) Woudenb. \& Crous, comb. nov. MycoBank MB803717.
Basionym: Ulocladium atrum Preuss, Linnaea 25: 75. 1852.
$\equiv$ Stemphylium atrum (Preuss) Sacc., Syll. Fungorum (Abellini) 4: 520. 1886.
Alternaria brassicae-pekinensis Woudenb. \& Crous, nom. nov. MycoBank MB803723.
Basionym: Ulocladium brassicae Yong Wang bis \& X.G. Zhang, Mycologia 100: 457. 2008, non Alternaria brassicae (Berk.) Sacc., 1880.
Etymology: Name refers to the host from which it was originally isolated.
Alternaria cantlous (Yong Wang bis \& X.G. Zhang) Woudenb. \& Crous, comb. nov. MycoBank MB803719.
Basionym: Ulocladium cantlous Yong Wang bis \& X.G. Zhang, Mycologia 102: 376. 2010.
Alternaria consortialis (Thüm.) J.W. Groves \& S. Hughes [as "consortiale"], Canad. J. Bot. 31: 636. 1953.
Basionym: Macrosporium consortiale Thüm., Herb. Mycol. Oecon. 9: no. 450. 1876.
$\equiv$ Stemphylium consortiale (Thüm.) J.W. Groves \& Skolko, Canad. J. Res., Sect. C, Bot. Sci.: 196. 1944.
$\equiv$ Pseudostemphylium consortiale (Thüm.) Subram., Curr. Sci. 30: 423. 1961.
$\equiv$ Ulocladium consortiale (Thüm.) E.G. Simmons, Mycologia 59: 84. 1967.
= Stemphylium ilicis Tengwall, Meded. Phytopathol. Lab. "Willie Commelin Scholten" 6: 44. 1924.

Alternaria cucurbitae Letendre \& Roum., in Roumeguère, Rev. Mycol. (Toulouse) 8 (no. 30): 93. 1886.
$\equiv$ Ulocladium cucurbitae (Letendre \& Roum.) E.G. Simmons, Mycotaxon 14: 48. 1982.

Alternaria heterospora Woudenb. \& Crous, nom. nov. MycoBank MB803724.
Basionym: Ulocladium solani Yong Wang bis \& X.G. Zhang, Mycol. Progr. 8: 209. 2009, non Alternaria solani Sorauer, 1896.
Etymology: Name refers to the various conidial morphologies observed during growth.
Alternaria multiformis(E.G. Simmons) Woudenb. \& Crous, comb. nov. MycoBank MB803720. Basionym: Ulocladium multiforme E.G. Simmons, Canad. J. Bot. 76: 1537. 1999 [1998].
Alternaria obovoidea (E.G. Simmons) Woudenb. \& Crous, comb. nov. MycoBank MB803721. Basionym: Ulocladium obovoideum E.G. Simmons, Mycotaxon 37: 104. 1990.
Alternaria subcucurbitae (Yong Wang bis \& X.G. Zhang) Woudenb. \& Crous, comb. nov. MycoBank MB803722.
Basionym: Ulocladium subcucurbitae Yong Wang bis \& X.G. Zhang, Mycologia 100: 456. 2008.

Alternaria terricola Woudenb. \& Crous, nom. nov. MycoBank MB803725.
Basionym: Ulocladium tuberculatum E.G. Simmons, Mycologia 59: 83. 1967, non Alternaria tuberculata M. Zhang \& T.Y. Zhang, 2006.
Etymology: Name refers to soil from which it was originally isolated.

Section Ulocladium (Preuss) Woudenb. \& Crous, comb. et stat. nov. MycoBank MB803747. Fig. 25.
Basionym: Ulocladium Preuss, Linnaea 24: 111. 1851.
Type species: Alternaria botrytis (Preuss) Woudenb. \& Crous.


Fig. 25. Alternaria sect. Ulocladium: conidia and conidiophores. A-B. A. capsici-annui. C-D. A. oudemansii. E-F. A. alternariae. G-H. A. botrytis. Scale bars $=10 \mu \mathrm{~m}$.

Diagnosis: Section Ulocladium is characterised by simple conidiophores, or with one or two short, geniculate, sympodial proliferations, with (mostly) single, obovoid, non-beaked conidia with a narrow base.

Notes: Section Ulocladium resembles sect. Ulocladioides. The epitype of Ulocladium, $U$. botrytis CBS 197.67, and the isotype of $U$. oudemansii (CBS 114.07) cluster with the Sinomyces representative, as do many other strains stored as $U$. botrytis in the CBS collection (data not shown). Furthermore, a strain stored as A. capsici-annui (CBS 504.74) in the CBS collection clusters within the Sinomyces clade and displays identical morphological features.

Alternaria alternariae (Cooke) Woudenb. \& Crous, comb. nov. MycoBank MB803716.
Basionym: Sporidesmium alternariae Cooke, Handb. Brit. Fungi 1: 1440. 1871.
$\equiv$ Stemphylium alternariae (Cooke) Sacc., Syll. Fungorum (Abellini) 4: 523. 1886.
$\equiv$ Ulocladium alternariae (Cooke) E.G. Simmons, Mycologia 59: 82. 1967.
$\equiv$ Sinomyces alternariae (Cooke) Yong Wang bis \& X.G. Zhang, Fungal Biol. 115: 194. 2011.

Alternaria botrytis (Preuss) Woudenb. \& Crous, comb. nov. MycoBank MB803718.
Basionym: Ulocladium botrytis Preuss, Linnaea 24: 111. 1851.
$\equiv$ Stemphylium botryosum var. ulocladium Sacc. (nom. nov.), Syll. Fungorum (Abellini) 4: 522. 1886.


Fig. 26. Alternaria sect. Undifilum: conidia and conidiophores. A-D. A. bornmuelleri. Scale bars $=10$ $\mu \mathrm{m}$.
$\equiv$ Stemphylium botryosum var. botrytis (Preuss) Lindau, Rabenhorst's. Kryptog.-Fl., Edn 2 (Leipzig) 1(9): 219. 1908.
Alternaria capsici-annui Săvul. \& Sandu, Hedwigia 75: 228. 1936.
Alternaria oudemansii (E.G. Simmons) Woudenb. \& Crous, comb. nov. MycoBank MB803715. Basionym: Ulocladium oudemansii E.G. Simmons, Mycologia 59: 86. 1967.

Section Undifilum (B.M. Pryor, Creamer, Shoemaker, McLain-Romero \& Hambl.) Woudenb. \& Crous, comb. et stat. nov. MycoBank MB803748. Fig. 26.
Basionym: Undifilum B.M. Pryor, Creamer, Shoemaker, McLain-Romero \& Hambl., Botany 87: 190. 2009.

Type species: Alternaria bornmuelleri (Magnus) Woudenb. \& Crous.
Diagnosis: Section Undifilum is characterised by ovate to obclavate to long ellipsoid, straight to inequilateral, single, transseptate conidia; septa can be thick, dark and rigid, and form unique germ tubes, which are wavy or undulate until branching. Species of this section occur on Fabaceae and almost all produce the toxic compound swaisonine.

Notes: Section Undifilum shares morphological features with section Embellisia, but is characterised by the formation of a wavy germ tube upon germination (Pryor et al. 2009). Based on previous studies, the swaisonine producing species U. oxytropis (Pryor et al. 2009, Lawrence et al. 2012), U. fulvum and U. cinereum (Baucom et al. 2012) also belong to this section, although the type species, $A$. bornmuelleri, does not produce swaisonine.

Alternaria bornmuelleri (Magnus) Woudenb. \& Crous, comb. nov. MycoBank MB803726.
Basionym: Helminthosporium bornmuelleri Magnus, Hedwigia 38 (Beibl.): 73. 1899.
$\equiv$ Undifilum bornmuelleri (Magnus) B.M. Pryor, Creamer, Shoemaker, McLain-Romero \& Hambl., Botany 87: 190. 2009.
Alternaria cinerea (Baucom \& Creamer) Woudenb. \& Crous, comb. nov. MycoBank MB803731.
Basionym: Undifilum cinereum Baucom \& Creamer, Botany 90: 872. 2012

Alternaria fulva (Baucom\& Creamer) Woudenb. \& Crous, comb. nov. MycoBank MB803732. Basionym: Undifilum fulvum Baucom \& Creamer, Botany 90: 871. 2012
Alternaria oxytropis (Q. Wang, Nagao \& Kakish.) Woudenb. \& Crous, comb. nov. MycoBank MB803727.
Basionym: Embellisia oxytropis Q. Wang, Nagao \& Kakish., Mycotaxon 95: 257. 2006.
$\equiv$ Undifilum oxytropis (Q. Wang, Nagao \& Kakish.) B.M. Pryor, Creamer, Shoemaker, McLain-Romero \& Hambl., Botany 87: 191. 2009.

## Monotypic lineages

The following six species are not assigned to one of the 24 above described Alternaria sections and are treated as separate, single species, lineages in this study. Future studies, including more and / or new Alternaria species, might eventually give rise to the formation of new sections, when these new species show to be closely related to one of these monotypic lineages.

Alternaria argyranthemi E.G. Simmons \& C.F. Hill, Mycotaxon 65: 32. 1997.
Alternaria brassicae (Berk.) Sacc., Michelia 2(no. 6): 129. 1880.
Basionym: Macrosporium brassicae Berk., Engl. Fl., Fungi (Edn 2) (London) 5: 339. 1836.
Additional synonyms listed in Simmons (2007).
Alternaria dennisii M.B. Ellis, Mycol. Pap. 125: 27. 1971.
$\equiv$ Embellisia dennisii (M.B. Ellis) E.G. Simmons, Mycotaxon 38: 257. 1990.
Alternaria helianthiinficiens E.G. Simmons, Walcz \& R.G. Roberts [as "helianthinficiens"], Mycotaxon 25: 204. 1986.
Alternaria soliaridae E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 374. 2007.
Alternaria thalictrigena K. Schub. \& Crous, Fungal Planet No. 12: 2. 2007.

Paradendryphiella Woudenb. \& Crous, gen. nov. MycoBank MB803750. Fig. 27.
Colonies on SNA effuse, entire, velvety, olivaceous. Reverse olivaceous-grey to iron-grey. Mycelium consisting of branched, septate hypha, (sub)hyaline, smooth. Conidiophores subhyaline, simple or branched, septate or not, straight or flexuous, often nodose with conspicuous, brown pigmentation at the apical region; at times reduced to conidiogenous cells. Conidiogenous cells terminal or lateral, with denticles aggregated at apex, with prominent conidial scars, thickened but not darkened; sometimes proliferating with a new head or a short, inconspicuous sympodial rachis. Conidia produced holoblastically, on narrow denticle, smooth, cylindrical to obclavate, straight or slightly flexuous, 1-7 transverse septa, pale to medium brown, often with dark septa (often constricted), and a darkened zone of pigmentation at the apex, and at the hilum, which is thickened, and somewhat protruding, with a minute marginal frill. Chlamydospores and sexual state not observed.

Type species: Paradendryphiella salina (G.K. Sutherl.) Woudenb. \& Crous.
Paradendryphiella salina (G.K. Sutherl.) Woudenb. \& Crous, comb. nov. MycoBank MB803751.
Basionym: Cercospora salina G.K. Sutherl., New Phytol. 15: 43. 1916.


Fig. 27. Paradendryphiella gen. nov.: conidia and conidiophores. A-B, D-E, G-I. P. salina. C, F. P. arenariae. Scale bars $=10 \mu \mathrm{~m}$.
$\equiv$ Dendryphiella salina (G.K. Sutherl.) Pugh \& Nicot, Trans. Brit. Mycol. Soc. 47(2): 266. 1964.
$\equiv$ Scolecobasidium salinum (G.K. Sutherl.) M.B. Ellis, More dematiaceous hyphomycetes (Kew): 192. 1976.
= Embellisia annulata de Hoog, Seigle-Mur., Steiman \& K.-E. Erikss., Antonie van Leeuwenhoek J. Microbiol. Serol. 51: 409. 1985.
Paradendryphiella arenariae (Nicot) Woudenb. \& Crous, comb. nov. MycoBank MB803752. Basionym: Dendryphiella arenariae Nicot, [as "arenaria"] Rev. Mycol. (Paris) 23: 93. 1958.
$\equiv$ Scolecobasidium arenarium (Nicot) M.B. Ellis, More dematiaceous hyphomycetes (Kew): 194. 1976.

## DISCUSSION

The well-supported node for the Alternaria clade obtained in the present study, and the low bootstrap support at the deeper nodes within the Alternaria complex is also consistently seen in previous phylogenetic studies published on these genera (Pryor \& Bigelow 2003, Inderbitzin et al. 2006, Pryor et al. 2009, Runa et al. 2009, Wang et al. 2011, Lawrence et al. 2012). The only phylogenetic study which displays a second fully supported node is based on a fivegene combined dataset of GAPDH, Alt a 1, actin, plasma membrane ATPase and calmodulin (Lawrence et al. 2013). This node, called clade A by the authors, supports eight "asexual" Alternaria species-groups and an Ulocladium (sect. Ulocladioides in our phylogenies) clade. By resolving these eight asexual phylogenetic lineages of Alternaria together with Ulocladium, which is sister to the sexual $A$. infectoria species-group and other sexual genera, Lawrence et al. (2013) elevated the asexual species-groups to sections within Alternaria. If we take this node as cut-off for the genus Alternaria in our phylogenies, this would leave an Alternaria clade with 14
internal clades (sections) and three monotypic lineages. In order to create a stable phylogenetic taxonomy, seven new genera need to be described of which three would be monotypic; $E$. dennissii, A. argyranthemi and A. soliaridae. Embellisia species would be assigned to five different genera of which four would be new, leaving only E. allii, E. chlamydospora and E. tellustris in the genus Embellisia. The well-known (medical) A. infectoria species-group would also have to be transferred to a new genus. This node is not supported in our study ( $0.98 \mathrm{PP} / 65$ ML Fig 1) and also the strict asexual / sexual division is not supported as two sexual morphs are found in section Panax. This approach would therefore give rise to multiple small genera, and would not end up in a logical and workable situation.

Based on our phylogenetic study on parts of the SSU, LSU, ITS, GAPDH, RPB2 and TEF1 gene regions of ex-type and reference strains of Alternaria species and all available allied genera, we resolved a Pleospora / Stemphylium-clade sister to Embellisia annulata, and a well-supported Alternaria clade. The Alternaria clade contains 24 internal clades and six monotypic lineages. In combination with a review of literature and morphology, the species within the Alternaria clade are all recognised here as Alternaria s. str. This puts the genera Allewia, Brachycladium, Chalastospora, Chmelia, Crivellia, Embellisia, Lewia, Nimbya, Sinomyces, Teretispora, Ulocladium, Undifilum and Ybotromyces in synonymy with Alternaria.

The support values for the different sections described in this study are plotted in a heatmap per gene / gene combination and phylogenetic method used (Table 2). This shows that the Bayesian method provides greater support than the Maximum Likelihood bootstrap support values, which is in congruence with previous reports (e.g. Douady et al. 2003). The sections Cheiranthus, Eureka and Nimbya have the lowest support values. For sect. Eureka this is mainly caused by the position of A. cumini, which clusters within sect. Embellisioides based on its RPB2 sequence and as a monotypic lineage based on its TEF1 sequence. Section Cheiranthus and Nimbya are small sections, with relative long branches. Future studies, including more strains and / or species in these sections, are necessary to check the stability of these long branches.

The sexual genus Crivellia with its Brachycladium asexual morph was described by Inderbitzin et al. (2006) with Crivellia papaveraceae (asexual morph Brachycladium penicillatum) as type species and B. papaveris, with an unnamed sexual morph, as second species. The genus Brachycladium, which was synonymised with Dendryphion (Ellis 1971), was resurrected for the non-sexual stage based on polyphyly within Dendryphion and morphological distinction from its type species, D. comosum. The type species of Brachycladium, B. penicillatum, resides in Alternaria sect. Crivellia, which places Brachycladium in synonymy with Alternaria instead of Dendryphion.

The genus Chalastospora was established by Simmons (2007) based on Chalastospora cetera, formerly Alternaria cetera. Two new Chalastospora species, C. ellipsoidea and C. obclavata, and $A$. malorum as $C$. gossypii were later added to the genus, based on sequence data of the ITS and LSU regions (Crous et al. 2009a). The genus is characterised by conidia which are almost always narrowly ellipsoid to narrowly ovoid with 1-6 transverse eusepta, generally lacking oblique or longitudinal septa (Crous et al. 2009a). Our study shows that Alternaria armoraciae and Embellisia abundans also belong to this clade. Juvenile conidia of $A$. armoraciae are ovoid, but vary from being narrow to broadly ovoid and ellipsoid, with 3-5 transverse septa and a single longitudinal septum in up to four of the transverse segments (Simmons 2007). Embellisia abundans was already mentioned as part of the Chalastospora clade (Andersen et al. 2009, Lawrence et al. 2012), and has long ovoid or obclavate conidia with 3-6 transverse septa and rarely any longitudinal septa (Simmons 1983). The description of
sect. Chalastospora does therefore not completely follow the original description of the genus Chalastospora.

The genus Embellisia is characterised by the thick, dark, rigid conidial septa and the scarcity of longitudinal septa (Simmons 2007). It was first described by Simmons (1971), with Embellisia allii as type and E. chlamydospora as second species. Multiple Embellisia species followed after the description of the genus, which was later linked to the sexual genus Allewia (Simmons 1990). The latest molecular-based revision was performed based on sequences of the GAPDH, ITS and Alt a 1 genes (Lawrence et al. 2012). They found that Embellisia split into four clades and multiple species, which clustered individually amidst Alternaria, Ulocladium or Stemphylium spp. Our results mostly support these data, but with the inclusion of more ex-type / representative strains of Alternaria some additions were made to the different Embellisia groups mentioned by Lawrence et al. (2012). Group I (sect. Embellisia) and III (sect. Embellisioides) are identical to the treatment of Lawrence et al. (2012) but group II (section Phragmosporae) and IV (section Eureka) are both expanded with four Alternaria species. As not all species from group II and IV display the typical morphological characters of Embellisia, we chose to name these Alternaria sections based on the oldest species residing in the respective sections. Embellisia abundans was already mentioned as being part of the Chalastospora-clade and E. indefessa formed a clade close to Ulocladium, which we now assign to sect. Cheiranthus. Embellisia dennisii also forms a separate lineage in our phylogenies; therefore the old name Alternaria dennissii is resurrected. Furthermore, the clustering of E. conoidea within the A. brassicicola species-group and E. annulata close to Stemphylium, now assigned as Paradendryphiella gen. nov., is confirmed by our phylogenetic data. The morphological character of thick, dark, rigid septa seems to have evolved multiple times and does not appear to be a valid character for taxonomic distinction at generic level.

The sexual morphs Lewia (Simmons 1986) and Allewia (Simmons 1990) were linked to Alternaria and Embellisia respectively, with the only difference between these genera being the morphology of their asexual morphs. Lewia chlamidosporiformans and L. sauropodis are transferred to the genus Leptosphaerulina (Simmons 2007), which leaves 11 Lewia species with a known Alternaria anamorph. Most of them (9/11) reside in sect. Infectoriae, the others are found in sect. Panax. Allewia only contains two species of which one resides in sect. Eureka and one in sect. Embellisioides. With the establishment of the new International Code of Nomenclature for algae, fungi and plants (ICN), the dual nomenclature system for sexual and asexual fungal morphs was abandoned and replaced by a single-name nomenclature (Hawksworth et al. 2011, Norvell 2011). In order to implement the new rules of the ICN, we synonymised Lewia and Allewia with Alternaria.

Although multiple molecular studies included Nimbya isolates in their phylogenies (Chou \& Wu 2002, Pryor \& Bigelow 2003, Hong et al. 2005a, Inderbitzin et al. 2006, Pryor et al. 2009), a more extensive molecular-based study was recently published by Lawrence et al. (2012). Based on sequences of the GAPDH, ITS and Alt a 1 genes, the authors found a Nimbya clade which contained the type species $N$. scirpicola together with $N$. scirpinfestans, $N$. scirpivora and $N$. caricis. The N. scirpicola isolate which we included in our study, was assigned to this genus by Simmons (1989) based on morphological characters, as is the one used in other molecular studies (Pryor \& Bigelow 2003, Hong et al. 2005a, Lawrence et al. 2012). The sequences of the ITS, GAPDH and Alt a 1 genes of these isolates are however not identical, but do cluster in the same clade in the two phylogenies (data not shown), together with the isolate of $N$. caricis. The $N$. gomphrenae isolate we included in our phylogeny was not representative of the name. Simmons mentioned in 1989 that Togashi (1926) described two different fungi and deposited the
small-spored species in the CBS collection, instead of the large-spored N. gomphrenae isolate. Nimbya gomphrenae CBS 108.27, which does not sporulate anymore, will therefore be treated as "Alternaria sp.", and resides in sect. Alternaria. The ITS sequence of N. gomphrenae from Chou \& $\mathrm{Wu}(2002)$ actually clusters within sect. Alternantherae. This section was described by Lawrence et al. (2012) and consists of three Nimbya species, which they renamed to Alternaria based on the position of the clade amidst the Alternaria species-groups. Based on the data from Chou \& Wu (2002), the name Alternaria gomphrenae is resurrected and placed in sect. Alternantherae.

The genus Sinomyces was described in by Wang et al. (2011) to accommodate Ulocladium alternariae and two new species from China, S. obovoideus and S. fusoides (type). The genus was differentiated from Ulocladium based on its simple conidiophores with a single apical pore or $1-2$ short, uniperforate, geniculate sympodial proliferations. Unfortunately, our DNA sequence analyses of the ex-type cultures of the two new species from China (CBS 124114 and CBS 123375) were not congruent with the GAPDH (both species) and Alt a 1 (S. obovoideus) sequences deposited in GenBank (data not shown), leading us to doubt the authenticity of these strains. This matter could not be resolved in spite of contacting the original depositors. The ex-type strain of $S$. alternariae (CBS 126989) was therefore included as representative of the genus Sinomyces. The presence of the epitype of Ulocladium, U. botrytis CBS 197.67, in this section resulted in us rejecting the name Sinomyces, and calling this sect. Ulocladium. In addition, the presence of $U$. oudemansii in this section, with conidiophores with $1-5$ uniperforate geniculations (Simmons 1967), also disagrees with the mentioned differentiation of Sinomyces from Ulocladium.

The type species of Ulocladium, $U$. botrytis, was typified by two representative strains QM 7878 (CBS 197.67) and QM 8619 (CBS 198.67) (Simmons 1967). Molecular studies performed afterwards showed that these strains are not identical (de Hoog \& Horré 2002). Most molecular studies performed used CBS 198.67 as representative of $U$. botrytis (Pryor \& Gilbertson 2000, Pryor \& Bigelow 2003, Hong et al. 2005a, Xue \& Zhang 2007, Pryor et al. 2009, Runa et al. 2009, Wang et al. 2010, Wang et al. 2011, Lawrence et al. 2012), which clusters in section Ulocladioides. However, de Hoog \& Horré (2002) epitypified U. botrytis with CBS 197.67, which clusters with Sinomyces strains, as does Ulocladium oudemansii, now named sect. Ulocladium. Extended phylogenetic analyses on all $U$. botrytis strains present in the CBS culture collection ( 16 isolates) also highlight this issue as they cluster either within sect. Ulocladium or sect. Ulocladioides (data not shown), both with one of the representative strains described by Simmons (1967). The suggestion to synonymise Ulocladium with Alternaria has been made several times in the past (Pryor \& Gilbertson 2000, Chou \& Wu 2002). The latest systematic revision of the genus Ulocladium (Runa et al. 2009) based on sequences from the ITS, GAPDH and Alt a 1 genes supported previous findings of poly- and paraphyletic relationships of Ulocladium among Alternaria, Embellisia and Stemphylium spp. (de Hoog \& Horré 2002, Pryor \& Bigelow 2003, Hong et al. 2005a). Ulocladium alternariae and $U$. oudemansii, now known as sect. Ulocladium, cluster separately. The core Ulocladium clade, containing the two sister clades now called sect. Ulocladioides and sect. Pseudoulocladium, was confirmed by later studies (Wang et al. 2010, Lawrence et al. 2012). Alternaria cheiranthi and Embellisia indefessa have been linked to Ulocladium (Pryor \& Gilbertson 2000, Pryor \& Bigelow 2003, Hong et al. 2005a, Pryor et al. 2009, Runa et al. 2009, Lawrence et al. 2012), but missed the diagnostic feature of Ulocladium. Our study showed that they form a sister section, sect. Cheiranthus, to sect. Ulocladioides. The confusing taxonomy in this genus strengthens our decision to reduce Ulocladium to synonymy with Alternaria. The characteristics of the former genus Ulocladium are added to the new broader Alternaria generic circumscription.

The genus Undifilum was described by Pryor et al. (2009) to accommodate the species $U$. oxytropis and $U$. bornmuelleri. It shares the morphological feature of thick, dark and rigid septa with the genus Embellisia, but was characterised by the formation of a wavy germ-tube upon germination (Pryor et al. 2009). A recent study on fungal endophytes in locoweeds in the US described two new Undifilum species (Baucom et al. 2012). Both new species produce the toxic compound swaisonine, which is also produced by U. oxytropis. Swaisonine is the cause of a neurological disease, locism, of grazing animals, resulting in economic losses in livestock (James \& Panter 1989). The production of swaisonine seems to be related to this section, although the type-species, $U$. bornmuelleri, does not produce this toxin.

The genus Ybotromyces contains one species, Y. caespitosus (originally Botryomyces caespitosus), which was isolated from a skin lesion of a human patient (de Hoog \& Rubio 1982). De Hoog et al. (1997) discovered a high similarity to Alternaria spp. based on restriction patterns of the ITS and SSU rDNA. A phylogeny study of melanised meristematic fungi based on their SSU and ITS rDNA sequences (Sterflinger et al. 1999) placed Y. caespitosus within the Pleosporales together with Alternaria and Pleospora. De Hoog \& Horré (2002) hypothesized that the ex-type strain of Y. caespitosus, CBS 177.80, is likely a synanamorph of a yet undescribed Alternaria species. Our phylogeny supports this hypothesis, and places the genus in sect. Infectoriae.

Chmelia slovaca, described from dermatic lesions of a human (Svobodová 1966), also clusters with sect. Infectoriae as was shown previously (de Hoog \& Horré 2002). The genus produces different types of chlamydospores and sporadically blastospores, but no conidia or conidiophores, which makes it difficult to identify based on morphology. De Hoog \& Horré (2002) were confident that Chmelia is a sterile member of $A$. infectoria, which is in agreement with our results.

## Genera unrelated to Alternaria

The placement of the sexual genus Pleospora (1863) with Stemphylium (1833) asexual morphs as basal sister clade to the Alternaria complex is well-documented in multiple molecular studies (Chou \& Wu 2002, Pryor \& Bigelow 2003, Hong et al. 2005a, Pryor et al. 2009, Lawrence et al. 2012). Therefore, we only included the type species of both genera in our phylogenies and used them as outgroup in the Alternaria phylogeny. Pleospora herbarum with its Stemphylium herbarum (CBS 191.86) asexual morph is the type species of the genus Pleospora. Stemphylium botryosum with its Pleospora tarda (CBS 714.68) sexual morph is the type species of the genus Stemphylium.

Embellisia annulata proved to be identical to the marine species Dendryphiella salina, and forms a well-supported clade in the Pleosporaceae together with D. arenariae. Several DNA-based studies (dela Cruz 2006, Jones et al. 2008, Zhang et al. 2009) concluded that the marine Dendryphiella species, D. arenariae and D. salina, belonged to the Pleosporaceae as sister clade to the Pleospora / Stemphylium complex. Furthermore, they showed the type species of Dendryphiella, D. vinosa, to be only distantly related, based on sequences of the ITS, SSU, LSU (Jones et al. 2008) and ITS, TEF1, RPB2 (dela Cruz 2006) gene regions. The transfer of the marine Dendryphiella species to Scolecobasidium (Ellis 1976), was also disputed. Scolecobasidium does not belong to the Pleosporales based on ITS, TEF1, and RPB2 sequences (dela Cruz 2006) and the morphology of the two Dendryphiella species does not fit the generic circumscription of Scolecobasidium (dela Cruz 2006, Jones et al. 2008). Ellis (1976) described denticles on the conidiogenous cells when the conidia become detached. However
other observers describe a marginal basal frill on the conidia after detachment, leaving a scar on the conidiophore. We propose to place the two species in the new genus Paradendryphiella as $C$. arenariae and $C$. salina. The need for a new genus to accommodate the two species was already suggested by Jones et al. (2008).

A recent study on Diademaceae, a family which is characterised by a flat circular operculum and bitunicate asci (Shoemaker \& Babcock 1992), excluded the sexual genera Comoclathris and Clathrospora, and (provisionally) placed them in the Pleosporaceae with alternaria-like asexual morphs (Zhang et al. 2011). Molecular data of two strains (Dong et al. 1998, Schoch et al. 2009) placed them within the Pleosporaceae. A confusing factor is that Dong et al. (1998) use the name Comoclathris baccata in their paper for strain CBS 175.52, but submitted their sequences under the name Clathrospora diplospora to GenBank. Shoemaker \& Babcock (1992) synonymised Clathrospora diplospora with Comoclathris baccata, which renders Comoclathris as the correct generic name. The confusion around these genera is illustrated by the fact that the CBS collection currently harbours six strains named as Clathrospora species of which four were renamed by Shoemaker \& Babcock in 1992 based on morphological studies, and three of these four strains were even transferred to the genus Comoclathris. The type species of Clathrospora, C. elynae is represented by two strains of which one, CBS 196.54, was also studied morphologically by Shoemaker and Babcock (1992). They form a well-supported clade, located basal to the Pleosporaceae (Fig. 2), outside the Alternaria complex. The type species of Comoclathris, Comoclathris lanata, was not available to us, but the two Comoclathris compressa strains cluster together in a well-supported clade within the Pleosporaceae, also outside the Alternaria complex, which we believe to be the correct phylogenetic placement of the genus. Two other strains, named Comoclathris magna (CBS 174.52) and Clathrospora heterospora (CBS 175.52) by Shoemaker and Babcock (1992), cluster amidst sect. Alternaria. Culture studies performed by Simmons (1952) showed the presence of alternaria-like conidia in these cultures and no (mature) ascospore formation. Presumably the species observed by Shoemaker and Babcock (1992) on plant material were lost during cultivation and became replaced by $A$. alternata species-group isolates. Both strains will be treated as "Alternaria sp."

The genus Alternariaster was first described by Simmons (2007) with Alternariaster helianthi, formerly Alternaria helianthi or Helminthosporium helianthi, as type and only species. It is distinct from Alternaria by the lack of a pigmented conspicuous internal, circumhilar ring in its conidia and conidiophores. Our study showed that this genus is clearly not part of the Alternaria complex and belongs to the Leptosphaeriaceae (Fig. 2) (Chapter 3).

In the recently published book "The genera of Hyphomycetes" (Seifert et al. 2011) three more genera are linked to Alternaria, namely Pantospora, Briansuttonia and Rhexoprolifer. A recent study on Pantospora included ITS and LSU sequence data of the type species Pantospora guazumae, which placed the genus in Mycosphaerellaceae (Minnis et al. 2011). This refutes the link with Alternaria. The genus Rhexoprolifer was described in 1996 by Matsushima with R. variabilis as type and only species, isolated from South Africa. Rhexoprolifer variabilis has rhexolytic conidial liberation and proliferating conidiophores with both phragmosporous and dictyosporous conidia. Briansuttonia was described in 2004 to accommodate Corynespora alternarioides (Castañeda Ruiz et al. 2004). The distoseptate muriform conidia of Briansuttonia do resemble Alternaria and Stemphylium, but the conidiogenous loci and euseptate conidia of Alternaria and holoblastic conidial ontogeny and euseptate muriform conidia of Stemphylium were enough for the authors to regard their taxon as a different genus. Both asexual genera presently lack molecular data, and we were unable to obtain any living specimens of these taxa. It would be valuable to include both genera in a
future study to resolve the connection among genera with muriform conidia and Alternaria.
The description of Alternaria s. str. in the present study is supported by i) a well-supported phylogenetic node in multiple analyses, ii) high similarity of clades within Alternaria based on SSU, LSU and ITS data, and iii) variation in the order of the clades between the different gene phylogenies, which is in congruence with low support values at these deeper nodes. We follow the precedence introduced by Lawrence et al. (2013) to assign the taxonomic status of sections of Alternaria for the different clades found, thus allowing us to retain the former generic names but associated with a different taxonomic status. For end-users, this also results in a more stable and understandable taxonomy and nomenclature.

## DEDICATION

We would like to dedicate this manuscript to the late Dr E.G. Simmons, who spent over 50 years of his life researching the systematics of the genus Alternaria. Without the time EGS spent on characterising the species included in this study, and his impeccable strain collection, which he placed in CBS for preservation and further study, the present study would not have been possible.

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## Reappraisal of the genus Alternariaster (Dothideomycetes)

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Key words: Alternaria, fungal pathogens, host-range, multi-gene phylogeny.


#### Abstract

Alternariaster was erected in 2007 to accommodate Alternaria helianthi, a fungal species known to cause leaf spots on Helianthus annuus (sunflower). It was segregated from Alternaria based on conidial morphology. Recently an unknown alternaria-like dematiaceous fungus was found associated with leaf spots on Bidens sulphurea (yellow cosmos) in Brazil. Based on a multi-gene phylogeny of parts of the ITS and LSU genes, this fungus was placed within the Leptosphaeriaceae with Alternariaster helianthi as its closest neighbour. Additional genes sequenced, RPB2 and GAPDH, confirmed this close relationship. The fungus on B. sulphurea has smaller conidia, $50-97.5 \times 12.5-20 \mu \mathrm{~m}$, compared to Al. helianthi, $80-160 \times 18-30 \mu \mathrm{~m}$, and lacks oblique or transverse septa which can be present in Al. helianthi. Pathogenicity studies on 18 plant species belonging to the Compositae showed that the B. sulphurea fungus only infected B. sulphurea, whereas Al. helianthi infected $H$. annuus and Galinsoga quadriradiata, a yet unreported host of $A l$. helianthi. The fungus causing disease on B. sulphurea is hence closely related but phylogenetically, morphologically and pathologically distinct from Al . helianthi, and therefore newly described as Alternariaster bidentis. The collection of a second species in the genus Alternariaster and the multigene phylogenetic analysis of these two species, confirmed Alternariaster to be a well-delimited genus in the Leptosphaeriaceae rather than the Pleosporaceae, to which Alternaria belongs.


## INTRODUCTION

The fungal genus Alternariaster was established by Simmons (2007) to accommodate Alternaria helianthi, a species known to cause leaf spots on Helianthus annuus (sunflower) worldwide (Alcorn \& Pont 1972, Ribeiro et al. 1974, Leite et al. 2007). This monotypic genus was segregated from Alternaria based on several morphological differences. Conidia of Alternariaster are not formed in chains, are cylindrical, ellipsoid or broadly ovoid, subhyaline to greyish brown, and only rarely form longitudinal or oblique septa. A fungus bearing significant morphological similarity to Alternariaster helianti was found on Bidens sulphurea in Brazil during studies of the pathogenic mycobiota of ornamentals.

Bidens sulphurea (Asteraceae) (common name yellow cosmos; in Brazil, cosmos-amarelo, aster-do-méxico and others), is a plant that is both regarded as a minor ornamental and as a weed, and appears in Brazil on published lists of ornamentals (Lorenzi \& Souza 2001) and weeds (Kissman \& Groth 1999, Lorenzi 2000). It is an annual herb, native to Mexico, which produces abundant showy yellow or orange flowers, and was probably introduced to Brazil as an ornamental, but became naturalised and invades rural areas, pastures and vegetable gardens. In 2004, a population of B. sulphurea was observed in the locality of Cristais in Viçosa (state of Minas Gerais, Brazil) in a garden and a nearby pasture bearing leaf spots, which eventually led to extensive blight and premature plant death. Only one published record of a fungal disease attacking B. sulphurea is known from Brazil, namely grey mold caused by Botrytis cinerea (Guatimosin et al. 2011). The leaf spot disease observed on B. sulphurea in 2004 was clearly dissimilar from grey mold. Samples were collected and examined on several occasions, and an alternaria-like dematiaceous hyphomycete was found to be associated with the disease. Elucidating the identity of this fungus was of relevance for the clarification of the etiology of the disease, and for the potential use of the fungus as a biocontrol agent of B. sulphurea. This contribution includes a description of a new fungal species as well as observations on its phylogenetic relationships and host range, together with a reappraisal of the genus Alternariaster.

## MATERIALS AND METHODS

## Samples and isolates

Representative samples of diseased specimens of Bidens sulphurea and Helianthus annuus were collected, dried in a plant press and deposited in the herbarium of the Universidade Federal de Viçosa (VIC). The fungi associated to the leaf spots on B. sulphurea and H. annuus were isolated in pure culture by direct transfer of spores onto plates containing vegetable broth-agar (VBA; Pereira et al. 2003) with a sterile fine pointed needle. Representative isolates of the fungi were deposited in the culture collection of the Universidade Federal de Viçosa (COAD) Brazil, and the CBS-KNAW Fungal Biodiversity Centre (CBS) The Netherlands (Table 1). The three Alternariaster helianthi strains present at the CBS, including the ex-type strain CBS 119672, were added to the study.

## Phylogeny

For DNA extraction pure cultures of the respective taxa were grown on potato-carrot agar (PCA; Crous et al. 2009c) for 7 d at $25^{\circ} \mathrm{C}$. Total genomic DNA of the isolates mentioned in Table 1
Table 1．Isolates used in this study and GenBank accession numbers for sequences．Bold accession numbers were generated in this study． GenBank accession numbers
GAPDH KC609333 KC609341 KC609347 KC609325
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KC609331
KC609332 KC609351
KC609352

| KC609334 | KC609342 |
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| KC609335 | KC584369 |
| KC609336 | KC609343 |
| KC609337 | KC584368 |
| KC609338 | KC609344 |
| KC609339 | KC609345 |
| KC609340 | KC609346 |

KC609346
JF740181 GQ387594 JF740183 GQ387611
 JF740187 JF740268
EU754153 JF740188 GQ387599 JF740190 GQ387601 JF740191 GQ387606 FJ427023 EU754187 JF740204 JF740281

Netherlands JF740206 JF740284

## Country

Brazil
Brazil
Unknown
Hungary
USA I！ze．g
Brazil Brazil Germany India Zimbabwe India Italy Zimbabwe Netherlands Netherlands
Bidens sulphurea
Bidens sulphurea Helianthus annuus Helianthus annuus Helianthus sp． Helianthus annuus
Helianthus annuus Helianthus annuus Quercus robur Dolichos biforus Glycine max Saline soil Chamaerops humilis Air Glycine max Berberis vulgaris Chenopodium album Chenopodium quinoa Lunaria annua
Rudbeckia sp． VIC $31814 ;$
COAD 364
VIC $31881 ;$
COAD 1191
IFO 9089

EGS 36.007
VIC $31838 ;$
COAD 1190
VIC $31926 ;$
COAD 1188
VIC $31927 ;$
COAD 1187
IMI 217262
IMI 113689；
ATCC 16207 PD 77／884 IMI 199777； ATCC 32813；
PD 74／56 PD 66／221
CBS 134021

| Alternariaster bidentis sp．nov．CBS 134021 |  |
| :--- | :--- |
|  | CBS 134185 |
| Alternariaster helianthi | CBS 327.69 | CBS 199.86 ZL96II SGD 8L0tをI SGつ

6I0t\＆I Sgつ CBS 134020 CBS 105.91 CBS 124140 CBS 124141 CBS 353.65 CBS 400.71 CBS 188.71 CBS 101636 CBS 363.93 CBS 448.68 $\infty$
$\stackrel{\infty}{\sim}$
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$\sim$
0 CBS 616.75 $99^{\circ}$ ItS SGつ Species name Alternariaster helianthi Coniothyrium carteri
Coniothyrium dolichi
Coniothyrium glycines
Coniothyrium multiporum
Coniothyrium palmarum
Coniothyrium telephii
Table 1. (Continued).

| Species name | CBS number ${ }^{1}$ | Other number ${ }^{1}$ | Host, substrate | Country | GenBank accession numbers |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | ITS | LSU | RPB2 | GAPDH |
| Leptosphaeria errabunda | CBS 617.75 | IMI 199775; ATCC 32814; PD 74/201 | Solidago sp. | Netherlands | JF740216 | JF740289 |  |  |
| Leptosphaeria etheridgei | CBS 125980 | DAOM 216539; <br> PD 95/1483 | Populus tremuloides | Canada | JF740221 | JF740291 |  |  |
| Leptosphaeria macrocapsa | CBS 640.93 | PD 78/139 | Mercurialis perennis | Netherlands | JF740237 | JF740304 |  |  |
| Leptosphaeria pedicularis | CBS 390.80 | PD 77/711 | Pedicularis sp. | Switzerland | JF740224 | JF740294 |  |  |
| Leptosphaeria rubefaciens | CBS 223.77 |  | Quercus sp. | Switzerland | JF740243 | JF740312 |  |  |
| Leptosphaeria scleroitoides | CBS 144.84 | $\begin{aligned} & \text { CECT 20025; } \\ & \text { PD 82/1061 } \end{aligned}$ | Medicago sativa | Canada | JF740192 | JF740269 |  |  |
| Leptosphaeria slovacica | CBS 389.80 | PD 79/171 | Balota nigra | Netherlands | JF740247 | JF740315 |  |  |
| Leptosphaeria sydowii | CBS 385.80 | PD 74/477 | Senecio jacobaea | UK | JF740244 | JF740313 |  |  |
| Leptosphaeria veronicae | CBS 145.84 | СЕСТ 20059; PD 78/273 | Veronica chamaedryoides | Netherlands | JF740254 | JF740320 |  |  |
| Paraleptosphaeria dryadis | CBS 643.86 |  | Dryas octopetala | Switzerland | JF740213 | GU301828 |  |  |
| Paraleptosphaeria macrospora | CBS 114198 | UPSC 2686 | Rumex domesticus | Norway | JF740238 | JF740305 |  |  |
| Paraleptosphaeria nitschkei | CBS 306.51 |  | Cirsium spinosissimum | Switzerland | JF740239 | JF740308 |  |  |
| Paraleptosphaeria orobanches | CBS 101638 | PD 97/12070 | Epifagus virginiana | USA | JF740230 | JF740299 |  |  |
| Paraleptosphaeria praetermissa | CBS 114591 |  | Rubus idaeus | Sweden | JF740241 | JF740310 |  |  |
| Phoma herbarum | CBS 615.75 |  | Rosa multiflora | Netherlands | FJ427022 | EU754186 |  |  |
| Plenodomus agnitus | CBS 121.89 | PD 82/903 | Eupatorium sp. | Netherlands | JF740194 | JF740271 |  |  |
| Plenodomus biglobosus | CBS 119951 |  | Brassica rapa | Netherlands | JF740198 | JF740274 |  |  |
| Plenodomus chrysanthemi | CBS 539.63 |  | Chrysanthemum sp. | Greece | JF740253 | GU238151 |  |  |
| Plenodomus collinsoniae | CBS 120227 | JCM 13073; <br> MAFF 239583 | Vitis coignetiae | Japan | JF740200 | JF740276 |  |  |
| Plenodomus confertus | CBS 375.64 |  | Anacyclus radiatus | Spain | AF439459 | JF740277 |  |  |
| Plenodomus congestus | CBS 244.64 |  | Erigeron canadensis | Spain | AF439460 | JF740278 |  |  |

Table 1. (Continued).

| Species name | CBS number ${ }^{1}$ | Other number ${ }^{1}$ | Host, substrate | Country | GenBank accession numbers |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | ITS | LSU | RPB2 | GAPDH |
| Plenodomus enteroleucus | CBS 142.84 | CECT 20063; PD 81/654 | Catalpa bignonioides | Netherlands | JF740214 | JF740287 |  |  |
| Plenodomus fallaciosa | CBS 414.62 | ETH 2961 | Satureja montana | France | JF740222 | JF740292 |  |  |
| Plenodomus hendersoniae | CBS 139.78 |  | Pyrus malus | Netherlands | JF740226 | JF740296 |  |  |
| Plenodomus influorescens | CBS 143.84 | $\begin{aligned} & \text { CECT 20064; } \\ & \text { PD 78/883 } \end{aligned}$ | Fraxinus excelsior | Netherlands | JF740228 | JF740297 |  |  |
| Plenodomus libanotidis | CBS 113795 | UPSC 2219 | Seseli libanotis | Sweden | JF740231 | JF740300 |  |  |
| Plenodomus lindquistii | CBS 381.67 |  | Helianthus annuus | Canada | JF740233 | JF740302 |  |  |
| Plenodomus lingam | CBS 260.94 | PD 78/989 | Brassica oleracea | Netherlands | JF740235 | JF740307 |  |  |
| Plenodomus lupini | CBS 248.92 | PD 79/141 | Lupinus mutabilis | Peru | JF740236 | JF740303 |  |  |
| Plenodomus pimpinellae | CBS 101637 | PD 92/41 | Pimpenella anisum | Israel | JF740240 | JF740309 |  |  |
| Plenodomus tracheiphilus | CBS 551.93 | PD 81/782 | Citrus limonia | Israel | JF740249 | JF740317 |  |  |
| Plenodomus visci | CBS 122783 | PD 74/1021 | Viscum album | France | JF740256 | EU754195 |  |  |
| Plenodomus wasabiae | CBS 120119 | FAU 559 | Eutrema wasabi | Taiwan | JF740257 | JF740323 |  |  |
| Pyrenochaeta cava | CBS 257.68 | IMI 331911 | Wheat field soil | Germany | JF740260 | EU754199 |  |  |
| Pyrenochaeta nobilis | CBS 407.76 |  | Laurus nobilis | Italy | EU930011 | EU754206 |  |  |
| Pyrenochaetopsis leptospora | CBS 101635 | PD 71/1027 | Secale cereale | Europe | JF740262 | GQ387627 |  |  |
| Pyrenochaetopsis pratorum | CBS 445.81 | PD 80/1254 | Lolium perenne | New Zealand | JF740263 | GU23816 |  |  |
| Subplenodomus apiicola | CBS 285.72 |  | Apium graveolens var. rapaceum | Germany | JF740196 | GU238040 |  |  |
| Subplenodomus drobnjacensis | CBS 269.92 | PD 88/896 | Eustoma exaltatum | Netherlands | JF740211 | JF740285 |  |  |
| Subplenodomus valerianae | CBS 630.68 | PD 68/141 | Valeriana phu | Netherlands | JF740251 | GU238150 |  |  |
| Subplenodomus violicola | CBS 306.68 |  | Viola tricolor | Netherlands | FJ427054 | GU238156 |  |  |

${ }^{1}$ ATCC: American Type Culture Collection, Virginia, USA; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CECT: Colección Española de Cultivos Tipo, Valencia University, Spain; COAD: Culture collection of the Universidade Federal de Viçosa, Brasil; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; EGS: Personal collection of Dr. E.G. Simmons; ETH: Swiss Federal Institute of Technology, Switzerland; FAU: Personal collection of Francis A. Uecker; IFO: Institute for Fermentation Culture Collection, Osaka, Japan; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; JCM: Japan Collection of Microorganisms, Riken Biosource Center, Japan; MAFF: MAFF GenBank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; PD: Plant Protection Service, Wageningen, The Netherlands; UPSC: Uppsala University Culture Collection, Sweden; VIC: herbarium of the Universidade Federal de Viçosa, Brasil.
was extracted using an Ultraclean microbial DNA isolation kit (Mobio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The primers V9G (de Hoog \& Gerrits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the ITS region, and LSU1fd (Crous et al. 2009b) and LR5 (Vilgalys \& Hester 1990) for the LSU region. The PCR conditions were as follows: $1 \mu \mathrm{LDNA}, 1 \times$ PCR buffer (Bioline GmbH, Luckenwalde, Germany), $40 \mu \mathrm{M}$ of each dNTP, $0.2 \mu \mathrm{M}$ of each primer, 0.25 units Taq polymerase (Bioline) and 1 mM (ITS) or 2 mM (LSU) $\mathrm{MgCl}_{2}$ in a final volume of $12.5 \mu \mathrm{~L}$. The amplification reactions were performed on a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). The initial denaturation step of $94{ }^{\circ} \mathrm{C}$ for 5 min was followed by 35 cycles of $94{ }^{\circ} \mathrm{C}(30 \mathrm{~s}), 48^{\circ} \mathrm{C}(30 \mathrm{~s})$, and $72^{\circ} \mathrm{C}(60 \mathrm{~s})$ and a final elongation step of $72^{\circ} \mathrm{C}(7 \mathrm{~min})$. The amplicons were sequenced in both directions using the same PCR primers and the BigDye ${ }^{\circledR}$ Terminator v. 1.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's recommendations. The products were analysed on an ABI Prism 3730 XL DNA Sequencer (Applied Biosystems). A consensus sequence was computed from the forward and reverse sequences using the Bionumerics v. 4.61 software package and deposited in GenBank (Table 1). The consensus regions of ITS and LSU were blasted against the NCBI Nucleotide collection database using Megablast to identify their closest neighbours. Hit sequences were downloaded and aligned using the multiple sequence alignment program MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html), and adjusted by eye where necessary. A Bayesian analysis was performed with MrBayes v. 3.2.1 (Huelsenbeck \& Ronquist 2001, Ronquist \& Huelsenbeck 2003) using a GTR model with gamma distributed rate variation for the single and concatenated gene regions. Further settings included a temperature value of 0.05 , sample frequency of 100 , for 5 M generations or when the average standard deviation of split frequencies dropped below 0.01 . The $50 \%$ majority rule consensus tree was calculated where the first $25 \%$ of sampled trees were discarded as 'burn-in'. The program Tracer v. 1.5.0 (Rambaut \& Drummond 2009) was used to ensure the convergence of the chains. Phylogenetic trees were visualised with Treeview v. 1.6.6 (Page 1996) and deposited in TreeBASE (www.treebase.org). The RPB2 and GAPDH sequences of the strains mentioned in Table 1 were also obtained and deposited in GenBank to confirm the close but distinct relationship of Alternariaster helianthi and the isolate from Bidens sulphurea.

## Taxonomy

Morphological characterisation of the isolates was done using fungal structures scraped from freshly infected leaves, and mounted in lactophenol or lactofuchsin on microscope slides and observed with an Olympus BX 51 light microscope fitted with a drawing tube and a digital camera (Olympus E330). Colony characteristics were noted after 14 d of growth on VBA and PCA at $25^{\circ} \mathrm{C}$, under a 12 h light regime. Colony colours were determined using the colour charts of Rayner (1970). Nomenclatural data were deposited in MycoBank (Crous et al. 2004).

## Pathogenicity studies

Fungal isolates were transferred to VBA plates and incubated for 14 d at $25^{\circ} \mathrm{C}$ under a 12 h light regime; light provided by two 40 W day-light fluorescent lamps and one 40 W NUV black-light lamp, placed 40 cm above the plates. After fungal colonies colonised the plates, 10 mL of sterile water was added to each plate and the surface of the plates was scraped with a rubber spatula. The resulting conidial suspension was adjusted to a concentration of $2 \times 10^{4}$ conidia $/ \mathrm{mL}$ with a haemocytometer. Twenty-day-old Bidens sulphurea plants, cultivated in individual pots, were

Chapter 3

Table 2. Pathogenicity results of Alternariaster bidentis (CBS 134021) and Al. helianthi (CBS 134018) on 18 plants belonging to the Asteraceae.

| Subfamily | Tribe | Species | ${\text { Al. } \text { bidentis }^{\mathbf{1}}}^{\text {Al. } \text { helianthi }^{\mathbf{1}}}$ |  |
| :--- | :--- | :--- | :---: | :---: |
| Cichorioideae | Cardueae | Cynara scolymus | - | n |
|  | Lactuceae | Lactuca sativa | - | n |
|  |  | Sonchus oleraceus | - | - |
| Asteroideae | Vernonia polyanthes | - | n |  |
|  | Mutisiae | Gerbera jamesonii | - | - |
|  | Astereae | Conyza canadensis | - | - |
|  | Anthemideae | Crysantemum morifolium | n | n |
|  | Eupatorieae | Mikania micrantha | - | - |
|  | Gnaphalieae | Helichrysum italicum | - | - |
|  | Helenieae | Tagetes minuta | - | - |
|  | Heliantheae | Bidens subalternans | - | - |
|  |  | Bidens sulphurea | + | - |
|  |  | Bidens pilosa | - | - |
|  |  | Dalia pinnata | - | - |
|  |  | Galinsoga quadriradiata | - | + |
|  |  | Helianthus annuus | - | + |
|  |  | Sphagneticola trilobata | - | - |
|  |  | Zinnia elegans | - | - |

${ }^{1}-=$ no symptoms; $+=$ leaf spot symptoms; $\mathrm{n}=$ necrosis.
sprayed until runoff with this conidial suspension. Each plant was covered with a transparent plastic bag wetted internally and left for 48 h with the base of the pots immersed in water in a greenhouse where temperature varied between $25-30^{\circ} \mathrm{C}$. Two plants were sprayed with sterile water and served as controls. After the 2 d period in the humid chamber, the plants were transferred to a bench in a greenhouse and observed daily for the appearance of disease symptoms.

A pathogenicity test was performed by separately inoculating the two isolates (B. sulphurea isolate CBS 134021 and Alternariaster helianthi CBS 134018) in duplo on individuals belonging to 18 plant species representing two subfamilies and nine tribes of the Asteraceae (Table 2). Plants inoculated were $30-60-\mathrm{d}$-old and $30-40 \mathrm{~cm}$ high. Whenever disease symptoms appeared observations were made under a dissecting microscope for the appearance of fungal structures. If necrosis of tissues appeared but no fungal structures were observed on such necrotic tissues after repeated observations, then fragments of these seemingly diseased tissues were removed, surface sterilized with sodium hypochlorite and plated on VBA plates to allow for possible isolation of the fungus.

## RESULTS

## Phylogeny

The ITS and LSU consensus sequences obtained for the B. sulphurea isolates and Alternariaster helianthi isolates showed a high level of identity to Plenodomus, Leptosphaeria and Para-


Fig. 1. Bayesian $50 \%$ majority rule consensus tree based on the ITS and LSU sequences of 61 strains. The Bayesian posterior probabilities (PP) of 0.95 and above are given at the nodes. Thickened lines indicate a PP of 1.0. The tree was rooted using Phoma herbarum (CBS 615.75).
leptosphaeria isolates (Leptosphaeriaceae) present in the NCBI nucleotide database. The closest relatives of our isolates were delineated in a study by de Gruyter et al. (2012). The alignment of the latter study was therefore used to construct a phylogenetic tree (Fig. 1, Table 1). Isolates from four families were included, with Phoma herbarum (CBS 615.75, Didymellaceae) as outgroup. The final alignment consisted of 61 taxa and 1425 characters (ITS 571, LSU 854), with 389 (ITS 288, LSU 101) unique site patterns. The Bayesian analysis resulted in 6451 trees per run, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated on a total of 9678 trees from two runs.

The eight Alternariaster isolates formed a well-supported clade (posterior probability of 1.0) between the genera Plenodomus and Heterospora within the Leptosphaeriaceae. The Alternariaster species formed two well-supported subclades within the Alternariaster clade. The RPB2 and GAPDH sequences showed $100 \%$ identity within the species, and $97 \%$ (881/908 nt) and $95 \%(561 / 593 \mathrm{nt})$ identity between species, which confirmed Al. helianthi and Al. bidentis as distinct species within the genus.

## Taxonomy

Alternariaster bidentis J.L. Alves \& R.W. Barreto, sp. nov. MycoBank MB800215. Fig. 2.
Etymology: Name refers to its host genus, Bidens.
Sexual morph unknown. Lesions on living leaves starting as broad, punctiform depressions on leaf blades and veins, becoming subcircular, yellowish brown and greyish centrally, up to 1 mm diam, surrounded by a halo of dark green tissue with a somewhat soaked appearance followed by a faint, yellow outer circular area; on leaf veins lesions elliptical to elongate, pale brown to purple; at later stages lesions coalescing and becoming flecked, subcircular up to 15 mm diam, leading to leaf blight and premature plant death. External mycelium indistinct. Internal mycelium composed of branched, septate, pale brown to greyish brown hyphae, $1.5-2.0 \mu \mathrm{~m}$ diam. Conidiophores hypophyllous, solitary or in groups of up to three, straight to slightly sinuous, $147.5-320 \times 10-12.5 \mu \mathrm{~m}$, simple to occasionally branched, $3-6$-septate, chestnut-brown at base, becoming yellowish brown at apex, smooth. Conidiogenous cells tretic, integrated, terminal to intercalary, sympodial, cylindrical, $25-165 \times 10-15 \mu \mathrm{~m}$; pale brown to yellowish. Conidiogenous loci conspicuous, $1-3$ per cell, protuberant, up to $5 \mu \mathrm{~m}$ diam, thickened and darkened. Conidia dry, solitary, cylindrical or subcylindrical, $50-97.5 \times 12.5-20$ $\mu \mathrm{m}$, apex and base obtusely rounded, 2-9 transversely septate (longitudinal or oblique septa absent), often deeply constricted at septa and larviform (in turgid freshly collected samples), eguttulate, subhyaline to greyish, smooth, hilum thickened and darkened, germinating both through apical and basal cells, occasionally also medially. Germ tubes oriented perpendicularly to the main axis of the conidium.

Culture characteristics: Relatively slow-growing (35-54 mm diam after 14 d ), colony raised centrally, cottony, white, with dark grey or brown outer zone (where sporulation is concentrated) and having a wide periphery of flat, sparse, greyish to brown mycelium, followed by an irregular dark grey rim. Spermogonia produced either with or without exposure to light, pycnidial, subglobose, $55-90 \times 50-80 \mu \mathrm{~m}$, walls of thick textura angularis. Spermatia subcylindrical, $6-12 \times 1-2 \mu \mathrm{~m}$, hyaline, smooth, germination not observed.

Specimens examined: Brazil, Minas Gerais, Viçosa, on living leaves of Bidens sulphurea, 21 Apr. 2004, R.W. Barreto (VIC 31814 - holotype, culture ex-type CBS 134021, COAD 364); Rio de Janeiro, Murineli, Duas Barras, on living leaves of B. sulphurea, 30 July 2011, R.W. Barreto (VIC 31883); Rio de Janeiro, Duas Barras, on living leaves of B. sulphurea, 4 Nov. 2011, R.W. Barreto (VIC 31884); Minas Gerais, Itabirito, São Gonçalo do Bação, on living leaves of $B$. sulphurea, 27 Jan. 2012, E. Guatimosim (CBS 134185, COAD 1191, VIC 31881); Minas Gerais, Itabirito, São Gonçalo do Bação, on living leaves of B. sulphurea, 7 Apr. 2012, E. Guatimosim (VIC 31882).


Fig. 2. Alternariaster bidentis. A. Flowering healthy plants of Bidens sulphurea. B. leaves with leaf spot and necrosis. C. Extensive blight. D-H. Conidia attached to conidiogenous cells. I. Spermogonium on SNA. Scale bars $=10 \mu \mathrm{~m}$, except $\mathrm{I}=100 \mu \mathrm{~m}$.

Alternariaster helianthi (Hansf.) E.G. Simmons, CBS Bio-diversity Ser. (Utrecht) 6: 667. 2007. MycoBank MB505050. Fig. 3.

Basionym: Helminthosporium helianthi Hansf., Proc. Linn. Soc. London 49. 1943 (1942-1943). = Alternaria helianthi (Hansf.) Tubaki \& Nishih., Trans. Brit. Mycol. Soc. 53: 148. 1969.

Sexual morph unknown. Lesions on living leaves starting as dispersed punctiform spots, occurring throughout the leaf blade, becoming subcircular to irregular in shape, yellowish, $3-11 \times 2-9 \mathrm{~mm}$, surrounded by a halo of dark green tissue, at later stages lesions coalesce, resulting in leaf blight and premature plant death. Conidiophores hypophyllous, solitary or in small groups, straight to slightly sinuous, $100-225 \times 7.5-10 \mu \mathrm{~m}$, simple, $3-6$-septate, pale to chestnut-brown, smooth. Conidiogenous cells tretic, integrated, terminal to intercalary, sympodial, cylindrical, 25-100 $\times$ $5-7.5 \mu \mathrm{~m}$, yellowish to pale brown. Conidiogenous loci conspicuous, $1-2$ per cell, protuberant, up to $5 \mu \mathrm{~m}$ diam, thickened and darkened. Conidia dry, solitary, cylindrical to subcylindrical, occasionally with cells of different size, $60-115 \times 11-29 \mu \mathrm{~m}$, apex and base rounded, transversally $5-9$ septate ( $1-2$ longitudinal or oblique septa), often deeply constricted at septa, eguttulate, subhyaline to pale brown, smooth, hilum thickened and darkened. Germ tubes orientated perpendicularly to the main axis of the conidium, and also polar.

Culture characteristics: On PCA and VBA, very slow-growing ( $8-11 \mathrm{~mm}$ diam after 14 d ). On PCA colony raised centrally, aerial mycelium felted, white, having a wide periphery of flat, sparse, olivaceous-buff to greenish glaucous mycelium, with irregular margins. On VBA colonies of dense cottony to velvety aerial mycelium, grey-olivaceous alternating with smoke-grey zones. In reverse olivaceous-buff centrally, and olivaceous at the edges on PCA, and grey-olivaceous alternating with olivaceous-black zones on VBA. Sporulation abundant. Spermagonia not observed.

Specimens examined: Brazil, Minas Gerais, Viçosa, on living leaves of Helianthus annuus, 30 May 2004 (COAD 302); Minas Gerais, Viçosa, on living leaves of H. annuus, 29 June 2010, J.L. Alves (CBS 134018, COAD 1190, VIC 31838); Minas Gerais, Belo Horizonte, on living leaves of H. annuus, 22 May 2012, J.L. Alves (CBS 134019, COAD 1188, VIC 31926); Minas Gerais, Viçosa, on living leaves of H. annuus, 25 May 2012, J.L. Alves (CBS 134020, COAD 1187, VIC 31927).

## Pathogenicity studies

The Al. bidentis isolate (CBS 134021) produced leaf spots only on B. sulphurea, whereas Al. helianthi (CBS 134018) produced leaf spots on H. annuus and also on Galinsoga quadriradiata (Table 2). Leaf necrosis appeared on four other species inoculated with Al. helianthi and one species when inoculated with Al. bidentis (Table 2), but no sporulation was observed on such necrotic tissues, and no fungal colonies were obtained from fragments of such tissues when plated on culture media.

## DISCUSSION

The genus Alternariaster was first described by Simmons (2007) with Alternariaster helianthi (formerly Alternaria helianthi and Helminthosporium helianthi) as type, and has hitherto been


Fig. 3. Alternariaster helianthi. A. Helianthus annuus with leaf spot and necrosis. B-E. Conidia. F-H. Conidia attached to conidiogenous cells. Scale bars $=10 \mu \mathrm{~m}$.
monotypic. The present phylogenetic analysis confirms Simmons's segregation of Alternariaster from Alternaria, by showing that Alternariaster is a well-delimited taxon belonging to the Leptosphaeriaceae (Fig. 1), instead of the Pleosporaceae to which Alternaria belongs (Schoch et al. 2009).

Initial attempts at identifying Alternariaster bidentis to the generic level based on morphological characters alone was challenging. Initially the fungus was regarded as a potential species of


Fig. 4. A, B. Alternariaster bidentis sp. nov. (CBS 134021) on Bidens sulphurea. A. Pathogenicity test evaluated at 14 d after inoculation (control left, inoculated right). B. Detail of necrosis. C. Alternariaster helianthi (CBS 134018) on Bidens sulphurea, no observed injury (control left, inoculated right). D, E. Alternariaster helianthi (CBS 134018) on H. annuus. D. Pathogenicity test evaluated at 4 d after inoculation (control left, inoculated right). E. detail of necrosis. F. Alternariaster bidentis sp. nov. (CBS 134021) on H. annuus, no observed injury (control left, inoculated right).

Alternaria. Nevertheless, as the fungus did not produce conidial chains, had conidia that appeared hyaline when young and when directly observed on leaves, were distinctly constricted at septa (having a larviform appearance) and were never found to have longitudinal or oblique septa. This combination of features suggested that it might be inadequately placed in Alternaria. However, the genus Alternaria contains some taxa noted for the absence of oblique and transverse septa, namely: A. chrysanthemi, A. thalictrina, A. thalictricola, and A. thalictrigena (Schubert et al. 2007a). Additionally, significant changes in conidial morphology were also observed when the fungus was grown in culture, particularly in older cultures where conidia became chestnut-brown and the formation of distosepta was observed at times. These features suggested that the species might belong to one of the genera segregated from Helminthosporium (Alcorn 1988), particularly Drechslera or Bipolaris. Alcorn (1991) separated Bipolaris, Drechslera and Exserohilum based on conidial germination patterns, septum ontogeny and their associated sexual morphs. Ironically, while the authors were trying to unravel the puzzle of the fungus occurring on Bidens sulphurea, the monograph on the genus Alternaria was published (Simmons 2007). In this monograph the genus Alternariaster was erected to accommodate Alternaria helianthi, a fungal species known to cause a serious disease of sunflower worldwide (Alcorn \& Pont 1972, Ribeiro et al. 1974, Leite et al. 2007). Alternariaster was segregated from Alternaria based on it being morphologically distinct by having cylindrical, ellipsoid or broad-ovoid in shape, subhyaline to greyish brown conidia not formed in chains and only rarely exhibiting longitudinal or oblique septa.

The morphology of Al. bidentis fits well into the concept proposed by Simmons for Alternariaster. However, this newly proposed species can be readily distinguished from Al. helianthi based on its conidial characters. Alternariaster bidentis has smaller conidia, 50-97.5 $\times 12.5-20 \mu \mathrm{~m}$, compared to Al. helianthi, $80-160 \times 18-30 \mu \mathrm{~m}$, without oblique or transverse septa, which though rare, could occur in Al. helianthi. Additionally spermogonia and spermatia were formed in cultures of Al. bidentis (but not in cultures of Al. helianthi) and were described here for the first time. Inoculations with Al. bidentis only resulted in leaf spots equivalent to those observed in the field on plants of B. sulphurea. Although necrosis appeared on leaves of Chrysanthemum morifolium, spots were limited to places where inoculum was deposited, and did not progress, nor could the fungus be re-isolated from such necrotic tissues. Necrosis was likely to be caused by one or more toxins produced by the fungus for which chrysanthemum was sensitive but not the other test plants. No leaf spot or necrosis of any kind appeared on Helianthus annuus inoculated with Al. bidentis or on B. sulphurea inoculated with Al. helianthi (Fig. 4). This is regarded as a complementary indication that $A l$. helianthi and Al. bidentis are distinct taxa. Inoculations of Al. helianthi (CBS 134018) led to typical Alternariaster leaf spots on $H$. annuus and Galinsoga quadriradiata after 5 d . Conidiophores and conidia could be identified as $A l$. helianthi on leaf spots on these two hosts after 7 d . Galinsoga quadriradiata is a new host for Al. helianthi. Alternariaster helianthi was previously reported to only infect $H$. annuus and Rudbeckia bicolor (Black-Eyed Susan) (Cho \& Shin 2004). Tissue necrosis was observed in Cynara scolymus, Chrysanthemum morifolium, Lactuca sativa and Vernonia polyanthes. As in the case of the inoculation of Al. bidentis on Chrysanthemum morifolium, it is likely that such necroses were a result of susceptibility of those hosts to one or more toxins produced by Al . helianthi. The delineation of a new Alternariaster species based on molecular, morphological and pathogenicity tests led to a reappraisal of the genus, with the conclusion that Alternariaster is a well-delimited genus belonging to the Leptosphaeriaceae, rather than to the Pleosporaceae, to which Alternaria belongs. The finding of this new taxon also confirmed a fortunate choice of name for the genus by Simmons, as this is also a fungus morphologically similar to Alternaria attacking a member of the Asteraceae.

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# Large-spored Alternaria pathogens in section Porri disentangled 

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Key words: Alternaria, early blight of potato, early blight of tomato, leaf and stem blight of sweet potato, multi-gene phylogeny, purple blotch of onion.


#### Abstract

The omnipresent fungal genus Alternaria was recently divided into 24 sections based on molecular and morphological data. Alternaria sect. Porri is the largest section, containing almost all Alternaria species with medium to large conidia and long beaks, some of which are important plant pathogens (e.g. Alternaria porri, A. solani and A. tomatophila). We constructed a multi-gene phylogeny on parts of the ITS, GAPDH, RPB2, TEF1 and Alt a 1 gene regions, which, supplemented with morphological and cultural studies, forms the basis for species recognition in sect. Porri. Our data reveal 63 species, of which 10 are newly described in sect. Porri, and 27 species names are synonymised. The three known Alternaria pathogens causing early blight on tomato all cluster in one clade, and are synonymised under the older name, A. linariae. Alternaria protenta, a species formerly only known as pathogen on Helianthus annuus, is also reported to cause early blight of potato, together with $A$. solani and $A$. grandis. Two clades with isolates causing purple blotch of onion are confirmed as $A$. allii and $A$. porri, but the two species cannot adequately be distinguished based on the number of beaks and branches as suggested previously. This is also found among the pathogens of Passifloraceae, which are reduced from four to three species. In addition to the known pathogen of sweet potato, $A$. bataticola, three more species are delineated of which two are newly described. A new Alternaria section is also described, comprising two large-spored Alternaria species with concatenate conidia.

Taxonomic novelties: New species - Alternaria alternariacida Woudenb. \& Crous, A. carthamicola Woudenb. \& Crous, A. catananches Woudenb. \& Crous, A. citrullicola Woudenb. \& Crous, A. conidiophora Woudenb. \& Crous, A. deserticola Woudenb. \& Crous, A. ipomoeae M. Truter, Woudenb. \& Crous, A. neoipomoeae M. Truter, Woudenb. \& Crous, A. paralinicola Woudenb. \& Crous, A. sennae Woudenb. \& Crous. New section in Alternaria - sect. Euphorbiicola Woudenb. \& Crous. Typifications (basionyms): Epitypifications - Alternaria bataticola W. Yamam., Cercospora crassa Sacc., Macrosporium porri Ellis, M. ricini Yoshii, Sporidesmium scorzonerae Aderh., Neotypification - Sporidesmium exitiosum var. dauci J.G. Kühn.


## INTRODUCTION

Alternaria is an important fungal genus with a worldwide distribution. This hyphomycetous ascomycete with phaeodictyospores includes saprophytic, endophytic and pathogenic species, which can be plant pathogens, post-harvest pathogens or human pathogens (Thomma 2003). The genus Alternaria was recently divided into 24 sections (Chapter 2) based on molecular and morphological data, which followed the recent initiative to divide Alternaria into sections (Lawrence et al. 2013). Alternaria sect. Porri is the largest section, containing almost all Alternaria species with medium to large conidia and long beaks. Among them are some important plant pathogens, such as Alternaria bataticola, A. porri, A. solani and A. tomatophila. Alternaria bataticola causes leaf petiole and stem blight of sweet potato in tropical and sub-tropical regions. The disease is most severe in East and Central Africa, with yield losses of over $70 \%$ reported (Osiru et al. 2007). Alternaria porri causes purple blotch of onion, a very destructive disease of onions worldwide. The disease causes a significant reduction in seed and bulb yield, with seed losses of up to $100 \%$ (Abo-Elyousr et al. 2014). Alternaria solani is the causative agent of early blight of potato. This very common disease, which can be found in most potato-growing countries, can cause considerable defoliation. The disease typically reduces yields by $\sim 20 \%$, but yield reductions of up to $80 \%$ have been reported (Horsfield et al. 2010). Alternaria tomatophila is known for causing early blight of tomato, attacking the leaves, stems and fruit. This airborne pathogen has spread worldwide, mainly affecting field crops. When left untreated the damage can result in plant defoliation in excess of $60 \%$ (Zitter \& Drennan 2005).

The identification of these species has been problematic for many years, with every large-spored Alternaria found on Solanaceae commonly being identified as A. solani. This assumption changed with the treatment of Alternaria species on Solanaceae, in which Simmons (2000) distinguished 22 Alternaria and Nimbya species on solanaceous hosts on the basis of morphology. On potato, Simmons described the large-spored, long-beaked species A. grandis and $A$. solani, while on tomato he described $A$. tomatophila, $A$. cretica and $A$. subcylindrica. The distinction between potato and tomato pathogens was supported by subsequent molecular studies and chemotaxonomy (Andersen et al. 2008, Rodrigues et al. 2010, Brun et al. 2013, Gannibal et al. 2014).

The taxonomy of Alternaria species on Allium is also confused. Macrosporium porri was first described as pathogen of Allium (Cooke \& Ellis 1879), followed by Alternaria allii (Nolla 1927). Both species were later synonymised (Angell 1929) and the name changed to Alternaria porri (Cifferi 1930). The name A. allii was resurrected by Simmons in his identification manual (2007) where he described five large-spored, long-beaked species from Allium, which he could distinguish based on morphology. Large-spored Alternaria from sweet potato were mostly identified as $A$. bataticola, even if the isolates from some studies (Osiru et al. 2008, Narayanin et al. 2010) showed morphological differences compared with the description of Simmons (2007).

In the present study we aim to use a molecular approach to delineate the medium- to largespored Alternaria species with long beaks in sect. Porri. A multi-locus analysis based on five partial gene regions, the internal transcribed spacer regions 1 and 2 and intervening 5.8S nrDNA (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), RNA polymerase second largest subunit (RPB2), translation elongation factor 1-alpha (TEF1) and the Alternaria major allergen gene (Alt a 1), was performed. All available ex-type and representative isolates of medium to large-spored, long-beaked species described in Simmons (2007) were included in this study. The present multi-locus analysis supplemented with morphological and cultural data forms the basis for species recognition in sect. Porri.

## MATERIALS AND METHODS

## Isolates

One hundred eighty-three Alternaria strains including 116 ex-type or representative strains present at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands were included in this study (Table 1). With "representative isolate" we refer to the strains used to describe the species based on morphology in Simmons (2007). Freeze-dried strains were revived in 2 mL malt / peptone ( $50 \% / 50 \%$ ) and subsequently transferred to oatmeal agar (OA, Crous et al. 2009c). Strains stored in the liquid nitrogen collection of the CBS were transferred to OA directly from the $-185^{\circ} \mathrm{C}$ storage.

## PCR and sequencing

DNA extraction was performed using the UltraClean Microbial DNA isolation kit (Mobio laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The ITS region was amplified with the primers V9G (De Hoog \& Gerrits van den Ende 1998) and ITS4 (White et al. 1990), the GAPDH region with gpd1 and gpd2 (Berbee et al. 1999) the RPB2 region with RPB2-5F2 (Sung et al. 2007) and fRPB2-7cR (Liu et al. 1999), the TEF1 gene with the primers EF1-728F and EF1-986R (Carbone \& Kohn 1999) or EF2 (O’Donnell et al. 1998) and the Alt a 1 region with the primers Alt-for and Alt-rev (Hong et al. 2005a). The ITS, GAPDH, RPB2 and TEF1 PCRs were performed as described in Chapter 2. The reaction mixture for the Alt a 1 PCR consisted of $1 \mu \mathrm{~L}$ genomic DNA, $1 \times \mathrm{NH}_{4}$ reaction buffer (Bioline, Luckenwalde, Germany), $3 \mathrm{mM} \mathrm{MgCl}{ }_{2}, 20 \mu \mathrm{M}$ of each dNTP, $0.2 \mu \mathrm{M}$ of each primer and 0.25 U BIOTAQ DNA polymerase (Bioline). Conditions for PCR amplification consisted of an initial denaturation step of 5 min at $94^{\circ} \mathrm{C}$ followed by 40 cycles of 30 s at $94^{\circ} \mathrm{C}, 30 \mathrm{~s}$ at $55^{\circ} \mathrm{C}$ and 60 s at $72^{\circ} \mathrm{C}$ and a final elongation step of 7 min at $72^{\circ} \mathrm{C}$. The PCR products were sequenced in both directions using the PCR primers and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and analysed with an ABI Prism 3730XL Sequencer (Applied Biosystems) according to the manufacturer's instructions. Consensus sequences were computed from forward and reverse sequences using the BioNumerics v. 4.61 software package (Applied Maths, St-Martens-Latem, Belgium). All newly generated sequences were deposited in GenBank (Table 1).

## Phylogenetic analysis

Multiple sequence alignments were generated with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/ server/index.html), and adjusted by eye where necessary. Bayesian inference and Maximum Likelihood analyses were performed on both the individual sequence datasets as well as the concatenated datasets as described in Chapter 2, with the sample frequency set to 1000 instead of 100 in the Bayesian analysis. For the TEF1 partition an online tool (http://www.hiv.lanl. gov/content/sequence/findmodel/findmodel.html) suggested the K2P model with a gamma-rate variation as nucleotide substitution model, and for the remaining four partitions the TrN model with gamma-distributed rate variation. Sequences from the type species of the phylogenetically closest section, sect. Gypsophilae, A. gypsophilae (Chapter 2), were used as outgroup. The resulting trees were printed with TreeView v. 1.6.6 (Page 1996) and the alignments and trees deposited into TreeBASE (http://www.treebase.org).

## Taxonomy

Cultures were incubated on potato-carrot agar (PCA, Crous et al. 2009c) and synthetic nutrient-poor agar (SNA, Nirenberg 1976) plates at moderate temperatures ( $\sim 22^{\circ} \mathrm{C}$ ) under CoolWhite fluorescent light with an 8 h photoperiod. After 7 d the growth rates were measured and the colony characters noted. Colony colours were rated according to Rayner (1970). Morphological descriptions were made for isolates grown on SNA with a small piece of autoclaved filter paper placed onto the agar surface to enhance sporulation. When sporulation occurred, the sellotape technique was used for making slide preparations (Schubert et al. 2007b) with Titan Ultra Clear Tape (Conglom Inc., Toronto, Canada) and Shear's medium as mounting fluid. The $95 \%$ confidence intervals were derived from measurements of 30 structures, with extremes given in parentheses. Photographs of characteristic structures were made with a Nikon Eclipse 80i microscope equipped with a Nikon digital sight DS-Fil high definition colour camera, using differential interference contrast (DIC) illumination and the Nikon software NIS-Elements D v. 3.00. Adobe Bridge CS5.1 and Adobe Photoshop CS5 Extended, v. 12.1, were used for the final editing and photographic preparation. Colonies which did not sporulate after 7 d were checked for sporulation up to 3 wk ; after this period they were noted as sterile. Nomenclatural data were deposited in MycoBank (Crous et al. 2004).

## RESULTS

## Phylogeny

Because the amplification / sequencing of the RPB2 region of CBS 137457 and the Alt a 1 region of CBS 119410 and CBS 117360 failed, these genes were included as missing data in the combined analysis of these isolates. The topologies of the trees obtained from the RAxML and Bayesian analyses were overall congruent, resulting in identical speciesclades. The phylogenies of the single-gene trees were congruent with one exception, CBS 137456, which swapped between clusters with the different genes used, resulting in a somewhat distorted picture in the combined analysis. The aligned sequences of the ITS ( 538 characters), GAPDH ( 581 characters), RPB2 ( 772 characters), TEF1 ( 355 characters) and Alt a 1 ( 476 characters) gene regions of the 183 included Alternaria strains had a total length of 2722 characters, with respectively $77,111,134,141$ and 131 unique site patterns. After discarding the burn-in phase trees, the Bayesian analysis resulted in 7502 trees from which the $50 \%$ majority rule consensus tree and posterior probabilities were calculated. The multi-gene phylogeny of section Porri (Fig. 1) divided the isolates in 62 species (clades) and one new Alternaria section. The species $A$. euphorbiicola and $A$. limicola, previously assigned to sect. Porri (Lawrence et al. 2013, Chapter 2), form a sister-clade to sect. Porri, here described as Alternaria sect. Euphorbiicola sect. nov. A Bayesian phylogeny based on the GAPDH, RPB2 and TEF1 sequences of representative isolates of the closely related sections in Alternaria (sequences obtained from Chapter 2) was constructed for comparison, with A. brassicicola CBS 118699 from sect. Brassisicola, as outgroup (Fig. 2).
Table 1. Isolates used in this study and their GenBank accession numbers. Bold accession numbers were generated in other studies.

| Species name | Strain number ${ }^{1,2}$ | Locality, host / substrate | GenBank accesion numbers |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | ITS | GAPDH | Alt a 1 | TEF1 | RPB2 |
| Alternaria acalyphicola | CBS 541.94; E.G.S. 38.100; IMI 266969 ${ }^{\text {T }}$ | Seychelles, Acalypha indica | KJ718097 | KJ717952 | KJ718617 | KJ718446 | KJ718271 |
| Alternaria agerati | CBS 117221; E.G.S. 30.001; QM $9369^{\text {R }}$ | USA, Ageratum houstonianum | KJ718098 | KJ717953 | KJ718618 | KJ718447 | KJ718272 |
| Alternaria agripestis | CBS 577.94; E.G.S. $41.034^{\text {T }}$ | Canada, Euphorbia esula | KJ718099 | JQ646356 | KJ718619 | KJ718448 | KJ718273 |
| Alternaria allii | CBS 107.28; E.G.S. $48.084^{T}$ (A. porri) | Puerto Rico, Allium cepa | KJ718100 | KJ717954 | KJ718620 | KJ718449 | KJ718274 |
|  | porri) <br> CBS 109.41; CBS 114.38 ( $A$. porri) | Denmark, Allium cepa | KJ718101 | KJ717955 | KJ718621 | KJ718450 | KJ718275 |
|  | CBS 225.76 (A. porri) | Italy, Allium porrum | KJ718102 | KJ717956 | KJ718622 | KJ718451 | KJ718276 |
|  | CBS 116701; E.G.S. 33.134 ${ }^{\text {R }}$ | USA, Allium cepa var. viviparum | KJ718103 | KJ717957 | KJ718623 | KJ718452 | KJ718277 |
|  | CBS 121345; E.G.S. 45.018 <br> (A. vanuatuensis ${ }^{\mathrm{T}}$ ) | Vanuatu, Allium cepa | KJ718104 | KJ717958 | KJ718624 | KJ718453 | KJ718278 |
| Alternaria alternariacida sp. nov. | CBS 105.51; ATCC 11078; IMI 46816; CECT $2997^{\text {T }}$ ( $A$. solani) | UK, Solanum lycopersicum | KJ718105 | KJ717959 | KJ718625 | KJ718454 | KJ718279 |
| Alternaria anagallidis | CBS 107.44 | Denmark, Anagallis arvensis | KJ718106 | JQ646338 | KJ718626 | EU130544 | KJ718280 |
|  | CBS 101004 | New Zealand, Anagallis arvensis | KJ718107 | KJ717960 | KJ718627 | KJ718455 | KJ718281 |
|  | CBS 117128; E.G.S. $42.074^{\text {R }}$ | New Zealand, Anagallis arvensis | KJ718108 | KJ717961 | KJ718628 | KJ718456 | KJ718282 |
|  | CBS 117129; E.G.S. 50.091 ${ }^{\text {R }}$ | New Zealand, Anagallis arvensis | KJ718109 | KJ717962 | KJ718629 | KJ718457 | KJ718283 |
| Alternaria anodae | PPRI 12376 | South Africa, Anoda cristata | KJ718110 | KJ717963 | KJ718630 | KJ718458 | KJ718284 |
| Alternaria aragakii | CBS 594.93; E.G.S. 29.016; QM $9046{ }^{\text {T }}$ | USA, Passiflora edulis | KJ718111 | KJ717964 | KJ718631 | KJ718459 | KJ718285 |
| Alternaria argyroxiphii | CBS 117222; E.G.S. 35.122 ${ }^{\text {² }}$ | USA, Argyroxiphium sp. | KJ718112 | JQ646350 | KJ718632 | KJ718460 | KJ718286 |
|  | PPRI 11848 | South Africa, Ipomoea batatas | KJ718113 | KJ717965 | KJ718633 | KJ718461 | KJ718287 |
|  | PPRI 11971 | South Africa, Ipomoea batatas | KJ718114 | KJ717966 | KJ718634 | KJ718462 | KJ718288 |
| Alternaria azadirachtae | CBS 116444; E.G.S. 46.195; BRIP 25386(ss1) ${ }^{\text {T }}$ | Australia, Azadirachta indica | KJ718115 | KJ717967 | KJ718635 | KJ718463 | KJ718289 |

Table 1. (Continued).
Alternaria bataticola

Alternaria calendulae
43.143; IMI 366164 ( $A$
heliophytonis ${ }^{\text {T }}$ )

| Species name | Strain number ${ }^{1,2}$ | Locality, host / substrate | GenBank accesion numbers |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | ITS | GAPDH | Alt a 1 | TEF1 | RPB2 |
|  | CBS 117091; E.G.S. 31.037 ${ }^{\text {R }}$ | USA, Carthamus tinctorius | KJ718133 | KJ717983 | KJ718651 | KJ718481 | KJ718307 |
| Alternaria carthamicola | CBS 117092; E.G.S. 37.057; <br> IMI 276943 ${ }^{\mathrm{T}}$ (A. carthami ${ }^{\mathrm{R}}$ ) | Iraq, Carthamus tinctorius | KJ718134 | KJ717984 | KJ718652 | KJ718482 | KJ718308 |
| Alternaria cassiae | CBS 478.81; E.G.S. $33.147^{\text {R }}$ | USA, Senna obtusifolia | KJ718135 | KJ717985 | KJ718653 | KJ718483 | KJ718309 |
|  | CBS 116119; E.G.S. 47.112; IMI 286317; IMI 392448 (A. sauropodis ${ }^{\mathrm{T}}$ ) | Malaysia, Sauropus androgynus | KJ718136 | KJ717986 | KJ718654 | KJ718484 | KJ718310 |
|  | CBS 117224; E.G.S. $40.121^{\text {R }}$ | Brazil, Senna obtusifolia | KJ718137 | KJ717987 | KJ718655 | KJ718485 | KJ718311 |
|  | CBS 117369; E.G.S. 50.166 <br> (A. hibiscinficiens ${ }^{\text {T }}$ ) | Fiji, Hibiscus sabdariffa | KJ718138 | KJ717988 | KJ718656 | KJ718486 | KJ718312 |
| Alternaria catananches sp. nov. | $\begin{aligned} & \text { CBS 137456; PD } \\ & 013 / 05703936^{\mathrm{T}} \end{aligned}$ | Netherlands, Catananche caerulea | KJ718139 | KJ717989 | KJ718657 | KJ718487 | KJ718313 |
| Alternaria centaureae | CBS 116446; E.G.S. $47.119^{\text { }}$ | USA, Centaurea solstitialis | KJ718140 | KJ717990 | KJ718658 | KJ718488 | KJ718314 |
| Alternaria cichorii | CBS 102.33; E.G.S. 07.017; QM $1760^{\text {T }}$ | Cyprus, Cichorium intybus | KJ718141 | KJ717991 | KJ718659 | KJ718489 | KJ718315 |
|  | CBS 117218; E.G.S. 52.046; IMI 225641 ${ }^{\text {R }}$ | Greece, Cichorium endivia | KJ718142 | KJ717992 | KJ718660 | KJ718490 | KJ718316 |
| Alternaria cirsinoxia | CBS 113261; E.G.S. $41.136^{\text {² }}$ | Canada, Cirsium arvense | KJ718143 | KJ717993 | KJ718661 | KJ718491 | KJ718317 |
| Alternaria citrullicola sp. nov. | CBS 103.32; VKM F-1881; Nattrass No. $190^{\mathrm{T}}$ ( $A$. cucumerina) | Cyprus, Citrullus vulgaris | KJ718144 | KJ717994 | KJ718662 | KJ718492 | KJ718318 |
| Alternaria conidiophora sp. nov. | CBS 137457 ${ }^{\text { }}$ | Netherlands, unknown | KJ718145 | KJ717995 | KJ718663 | KJ718493 |  |
| Alternaria crassa | CBS 103.18 | USA, Datura sp. | KJ718146 | KJ717996 | KJ718664 | KJ718494 | KJ718319 |
|  | CBS 110.38 ${ }^{\text {T }}$ | Cyprus, Datura stramonium | KJ718147 | KJ717997 | KJ718665 | KJ718495 | KJ718320 |
|  | CBS 109160; E.G.S. 45.075; IMI 262408; IMI 381021 (A. capsici ${ }^{\text {T }}$ ) | Australia, Capsicum annuum | KJ718148 | AY562408 | AY563298 | KJ718496 | KJ718321 |
|  | CBS 109162; E.G.S. 46.014 | USA, Nicandra physalodes | KJ718149 | GQ180073 | GQ180089 | KJ718497 | KJ718322 |


| Species name | Strain number ${ }^{1,2}$ | Locality, host / substrate | GenBank accesion numbers |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | ITS | GAPDH | Alt a 1 | TEF1 | RPB2 |
| Alternaria cucumerina | CBS 116647; E.G.S. $46.013^{\text {R }}$ | USA, Datura stramonium | KJ718150 | KJ717998 | KJ718666 | KJ718498 | KJ718323 |
|  | CBS 116648; E.G.S. $50.180^{\text {R }}$ | New Zealand, Datura stramonium | KJ718151 | KJ717999 | KJ718667 | KJ718499 | KJ718324 |
|  | CBS 122590; E.G.S. $44.071^{\text {R }}$ | USA, Datura stramonium | KJ718152 | GQ180072 | GQ180088 | KJ718500 | KJ718325 |
|  | CBS 116114; E.G.S. 35.123 <br> (A. loofahae ${ }^{\text {T }}$ ) | USA, Luffa acutangula | KJ718153 | KJ718000 | KJ718668 | KJ718501 | KJ718326 |
|  | CBS 117225; E.G.S. $41.127^{\text {R }}$ | USA, Cucumis melo | KJ718154 | KJ718001 | KJ718669 | KJ718502 | KJ718327 |
|  | CBS 117226; E.G.S. 44.197; BRIP $23060^{\text {R }}$ | Australia, Cucumis melo | KJ718155 | KJ718002 | KJ718670 | KJ718503 | KJ718328 |
| Alternaria cyamopsidis | CBS 364.67; E.G.S. 17.065; QM $8575^{\text {R }}$ | USA, Cyamopsis tetragonoloba | KJ718156 | KJ718003 | KJ718671 | KJ718504 | KJ718329 |
|  | CBS 117219; E.G.S. 13.120; QM $8000^{\text {R }}$ | USA, Cyamopsis tetragonoloba | KJ718157 | KJ718004 | KJ718672 | KJ718505 | KJ718330 |
| Alternaria dauci | CBS 111.38 ${ }^{\text {T }}$ | Italy, Daucus carota | KJ718158 | KJ718005 | KJ718673 | KJ718506 | KJ718331 |
|  | CBS 106.48 | Unknown, Daucus carota | KJ718159 | KJ718006 | KJ718674 | KJ718507 | KJ718332 |
|  | CBS 345.79; LEV 14814 | New Zealand, Daucus carota | KJ718160 | KJ718007 | KJ718675 | KJ718508 | KJ718333 |
|  | CBS 477.83; CBS 721.79; PD 79/954 (A. cichorii) | Netherlands, Cichorium intybus var. foliosum | KJ718161 | KJ718008 | KJ718676 | KJ718509 | KJ718334 |
|  | CBS 101592 | Netherlands, Daucus carota | KJ718162 | KJ718009 | KJ718677 | KJ718510 | KJ718335 |
|  | CBS 117097; E.G.S. $46.006^{\text {R }}$ | USA, Daucus carota | KC584192 | KC584111 | KJ718678 | KC584651 | KC584392 |
|  | CBS 117098; E.G.S. $46.152^{\text {R }}$ | New Zealand, Daucus carota | KJ718163 | KJ718010 | HE796726 | KJ718511 | KJ718336 |
|  | CBS 117099; E.G.S. $47.131^{\text {R }}$ | USA, Daucus carota | KJ718164 | KJ718011 | KJ718679 | KJ718512 | KJ718337 |
|  | CBS 117100; E.G.S. 47.138 <br> (A. poonensis ${ }^{\mathrm{R}}$ ) | Puerto Rico, Coriandrum sativum | KJ718165 | JQ646348 | KJ718680 | KJ718513 | KJ718338 |
| Alternaria deserticola sp. nov. | CBS $110799^{\mathrm{T}}$ ( $A$. acalyphicola) | Namibia, desert soil | KJ718249 | KJ718077 | KJ718755 | KJ718595 | KJ718424 |
| Alternaria dichondrae | CBS 199.74; E.G.S. $38.007^{\text {T }}$ | Italy, Dichondra repens | KJ718166 | JQ646357 | JQ646441 | KJ718514 | KJ718339 |
|  | CBS 200.74; E.G.S. $38.008^{\text {T }}$ | Italy, Dichondra repens | KJ718167 | KJ718012 | KJ718681 | KJ718515 | KJ718340 |


| Species name | Strain number ${ }^{1,2}$ | Locality, host / substrate | GenBank accesion numbers |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | ITS | GAPDH | Alt a 1 | TEF1 | RPB2 |
| Alternaria echinaceae | CBS 346.79 | New Zealand, Dichondra repens | KJ718168 | KJ718013 | KJ718682 | KJ718516 | KJ718341 |
|  | CBS 117127; E.G.S. $40.057^{\text {R }}$ | New Zealand, Dichondra sp. | KJ718169 | KJ718014 | KJ718683 | KJ718517 | KJ718342 |
|  | CBS 116117; E.G.S. $46.081^{\text {T }}$ | New Zealand, Echinacea sp. | KJ718170 | KJ718015 | KJ718684 | KJ718518 | KJ718343 |
|  | CBS 116118; E.G.S. $46.082^{\text {R }}$ | New Zealand, Echinacea sp. | KJ718171 | KJ718016 | KJ718685 | KJ718519 | KJ718344 |
| Alternaria grandis | CBS 109158; E.G.S. $44.106^{\text {² }}$ | USA, Solanum tuberosum | KJ718239 | JQ646341 | JQ646425 | EU130547 | KJ718414 |
|  | CBS 116695; E.G.S. $44.108^{\text {R }}$ | USA, Solanum tuberosum | KJ718241 | KJ718070 | KJ718748 | KJ718587 | KJ718416 |
| Alternaria euphorbiicola | CBS 198.86; E.G.S. 38.082 | USA, Euphorbia pulcherrima | KJ718172 | KJ718017 | KJ718686 | KJ718520 | KJ718345 |
|  | CBS 119410; E.G.S. $41.029^{\text {R }}$ | USA, Euphorbia pulcherrima | KJ718173 | KJ718018 |  | KJ718521 | KJ718346 |
|  | CBS 133874; E.G.S. 38.191 | USA, Euphorbia hyssopifolia | KJ718174 | KJ718019 | KJ718687 | KJ718522 | KJ718347 |
| Alternaria gypsophilae | CBS 107.41; E.G.S. 07.025; IMI $264349^{\text {T }}$ | Netherlands, Gypsophila elegans | KC584199 | KC584118 | KJ718688 | KC584660 | KC584401 |
| Alternaria ipomoeae sp. nov. | CBS 219.79 ${ }^{\text {T }}$ ( . cucumerina) | Ethiopia, Ipomoea batatas | KJ718175 | KJ718020 | KJ718689 | KJ718523 | KJ718348 |
|  | PPRI 8988 | South Africa, Ipomoea batatas | KJ718176 | KJ718021 | KJ718690 | KJ718524 | KJ718349 |
| Alternaria jesenskae | CBS 133855; CCM $8361{ }^{\text {T }}$ | Slovakia, Fumana procumbens | KJ718177 | KJ718022 | KJ718691 | KJ718525 | KJ718350 |
| Alternaria limicola | CBS 483.90; E.G.S. 39.070 ${ }^{\text {T }}$ | Mexico, Citrus aurantiifolia | KJ718178 | JQ646329 | JQ646413 | KJ718526 | KJ718351 |
|  | CBS 117360; E.G.S. $43.009^{\text {R }}$ | Mexico, Citrus sp. | KJ718179 | KJ718023 |  | KJ718527 | KJ718352 |
| Alternaria linariae | CBS 105.41; E.G.S. $07.016^{\text {T }}$ | Denmark, Linaria maroccana | KJ718180 | KJ718024 | KJ718692 | KJ718528 | KJ718353 |
|  | CBS 108.53 (A. solani) | Unknown, unknown | KJ718181 | KJ718025 | KJ718693 | KJ718529 | KJ718354 |
|  | CBS 107.61 (A. solani) | Belgium, unknown | KJ718182 | KJ718026 | KJ718694 | KJ718530 | KJ718355 |
|  | CBS 109156; E.G.S. 42.156 (A. tomatophila ${ }^{\text {T }}$ ) | USA, Solanum lycopersicum | KJ718183 | JQ646347 | GQ180101 | KJ718531 | KJ718356 |
|  | CBS 109161; E.G.S. 45.113 <br> (A. subcylindrica ${ }^{\mathrm{T}}$ ) | USA, Solanum lycopersicum var. cerasiforme | KJ718184 | JQ646345 | JQ646429 | KJ718532 | KJ718357 |
|  | CBS 109164; E.G.S. 46.188 (A. cretica ${ }^{\text {T }}$ ) | Greece, Solanum lycopersicum | KJ718185 | JQ646342 | JQ646426 | EU130545 | KJ718358 |
|  | CBS 116438; E.G.S. 41.057 <br> (A. cucumericola ${ }^{\mathrm{T}}$ ) | New Zealand, Cucumis sativus | KJ718186 | KJ718027 | KJ718695 | KJ718533 | KJ718359 |


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| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | ITS | GAPDH | Alt a 1 | TEF1 | RPB2 |
| Alternaria macrospora | CBS 116441; E.G.S. 45.108 (A. tabasco ${ }^{\mathrm{T}}$ ) | USA, Capsicum frutescens | KJ718187 | KJ718028 | KJ718696 | KJ718534 | KJ718360 |
|  | CBS 116704; E.G.S. 44.074 <br> (A. tomatophila ${ }^{\mathrm{R}}$ ) | USA, Solanum lycopersicum | KJ718188 | KJ718029 | KJ718697 | KJ718535 | KJ718361 |
|  | CPC 21620 | Thailand, Solanum lycopersicum | KJ718189 | KJ718030 | KJ718698 | KJ718536 | KJ718362 |
|  | CBS 106.29 (A.porri) | Nigeria, Gossypium sp. | KJ718193 | KJ718032 | KJ718701 | KJ718540 | KJ718366 |
|  | CBS 117228; E.G.S. 50.190; ATCC 58172 ${ }^{\text {T }}$ | USA, Gossypium barbadense | KC584204 | KC584124 | KJ718702 | KC584668 | KC584410 |
| Alternaria montanica | CBS 121343; E.G.S. 44.112; IMI $257563^{\text {T }}$ | USA, Cirsium arvense | KJ718194 | KJ718033 | KJ718703 | KJ718541 | KJ718367 |
| Alternaria multirostrata | CBS 712.68; ATCC 18515; IMI 135454; MUCL 11722 ;QM 8820; VKM F-2997 ${ }^{\text {² }}$ | USA, Richardia scabra | KJ718195 | JQ646362 | KJ718704 | EU130546 | KJ718368 |
| Alternaria neoipomoeae sp. nov. | CBS 713.68; ATCC 18517; IMI 135455; MUCL 11715; QM $8821^{\text {R }}$ | USA, Richardia scabra | KJ718196 | KJ718034 | KJ718705 | KJ718542 | KJ718369 |
|  | PPRI 8990 | South Africa, Ipomoea batatas | KJ718197 | KJ718035 | KJ718706 | KJ718543 | KJ718370 |
|  | PPRI 11845 ${ }^{\text {T }}$ | South Africa, Ipomoea batatas | KJ718198 | KJ718036 | KJ718707 | KJ718544 | KJ718371 |
|  | PPRI 11847 | South Africa, Ipomoea batatas | KJ718199 | KJ718037 | KJ718708 | KJ718545 | KJ718372 |
|  | PPRI 13903 | South Africa, Ipomoea batatas | KJ718200 | KJ718038 | KJ718709 | KJ718546 | KJ718373 |
| Alternaria nitrimali | CBS 109163; E.G.S. $46.151^{\text {T }}$ | Puerto Rico, Solanum viarum | KJ718201 | JQ646358 | KJ718710 | KJ718547 | KJ718374 |
| Alternaria novae-guineensis | CBS 116120; E.G.S. $47.198^{\text {T }}$ | Papua New Guinea, Citrus sp. | KJ718202 | KJ718039 | KJ718711 | KJ718548 | KJ718375 |
|  | PPRI 12171 | South Africa, Galinsoga parviflora | KJ718203 | KJ718040 | KJ718712 | KJ718549 | KJ718376 |
| Alternaria obtecta | CBS 117367; E.G.S. $42.063^{\text {R }}$ | USA, Euphorbia pulcherrima | KJ718204 | KJ718041 | KJ718713 | KJ718550 | KJ718377 |
|  | CBS 134278; E.G.S. 42.064 | USA, Euphorbia pulcherrima | KJ718205 | KJ718042 | KJ718714 | KJ718551 | KJ718378 |
| Alternaria paralinicola sp. nov. | CBS 116652; E.G.S. 47.157; DAOM $225747^{\mathrm{T}}$ ( A. linicola $^{\mathrm{R}}$ ) | Canada, Linum usitatissimum | KJ718206 | KJ718043 | KJ718715 | KJ718552 | KJ718379 |
| Alternaria passiflorae | CBS 113.38 | Australia, Passifora edulis | KJ718207 | JQ646353 | JQ646437 | KJ718553 | KJ718380 |


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|  |  |  | ITS | GAPDH | Alt a 1 | TEF1 | RPB2 |
| Alternaria pipionipisi | CBS 166.77 (A. solani) | New Zealand, Capsicum frutescens | KJ718208 | KJ718044 | KJ718716 | KJ718554 | KJ718381 |
|  | CBS 629.93; E.G.S. 16.150; QM $8458^{\text {R }}$ | New Zealand, Passiflora edulis | KJ718209 | KJ718045 | KJ718717 | KJ718555 | KJ718382 |
|  | CBS 630.93; E.G.S. 29.020; <br> QM 9050 (A. hawaiiensis ${ }^{\text {T }}$ ) | USA, Passiflora edulis | KJ718210 | JQ646352 | KJ718718 | KJ718556 | KJ718383 |
|  | CBS 116333; E.G.S. 50.121 <br> (A. gaurae ${ }^{\mathrm{T}}$ ) | New Zealand, Gaura lindheimeri | KJ718211 | KJ718046 | KJ718719 | KJ718557 | KJ718384 |
|  | CBS 117102; E.G.S. $51.165^{\text {R }}$ | New Zealand, Passiflora ligularis | KJ718212 | KJ718047 | KJ718720 | KJ718558 | KJ718385 |
|  | CBS 117103; E.G.S. $52.032^{\text {R }}$ | New Zealand, Passiflora caerulea | KJ718213 | KJ718048 | KJ718721 | KJ718559 | KJ718386 |
|  | CBS 116115; E.G.S. 40.096; IMI $340950^{\text {T }}$ | India, Cajanus cajan | KJ718214 | KJ718049 | KJ718722 | KJ718560 | KJ718387 |
|  | CBS 117365; E.G.S. 42.048 <br> (A. obtecta ${ }^{\mathrm{R}}$ ) | USA, Euphorbia pulcherrima | KJ718215 | KJ718050 | KJ718723 | KJ718561 | KJ718388 |
|  | CBS 134265; E.G.S. 42.047 <br> (A. obtecta) | USA, Euphorbia pulcherrima | KJ718216 | KJ718051 | KJ718724 | KJ718562 | KJ718389 |
| Alternaria porri | CBS 116649; E.G.S. 17.082; QM 8613 (A. allii ${ }^{\mathrm{R}}$ ) | USA, Allium cepa | KJ718217 | KJ718052 | KJ718725 | KJ718563 | KJ718390 |
|  | CBS 116698; E.G.S. $48.147^{\text {R }}$ | USA, Allium cepa | DQ323700 | KC584132 | KJ718726 | KC584679 | KC584421 |
|  | CBS 116699; E.G.S. $48.152^{\text {² }}$ | USA, Allium cepa | KJ718218 | KJ718053 | KJ718727 | KJ718564 | KJ718391 |
| Alternaria protenta | CBS 347.79; E.G.S. 44.091; LEV 14726; ATCC 38569 (A. solani) | New Zealand, Solanum lycopersicum | KJ718219 | KJ718054 | KJ718728 | KJ718565 | KJ718392 |
|  | CBS 116437; E.G.S. 32.076 <br> (A. hordeiseminis ${ }^{\mathrm{T}}$ ) | New Zealand, Hordeum vulgare | KJ718220 | KJ718055 | KJ718729 | KJ718566 | KJ718393 |
|  | CBS 116651; E.G.S. 45.020 (A. solani ${ }^{R}$ ) | USA, Solanum tuberosum | KC584217 | KC584139 | GQ180097 | KC584688 | KC584430 |


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|  |  |  | ITS | GAPDH | Alt a 1 | TEF1 | RPB2 |
|  | CBS 116696; E.G.S. 45.023; IMI 372955R | Israel, Helianthus annuus | KJ718221 | JQ646335 | JQ646419 | KJ718567 | KJ718394 |
|  | CBS 116697; E.G.S. 45.024; <br> IMI 372957R | Israel, Helianthus annuus | KJ718222 | KJ718056 | KJ718730 | KJ718568 | KJ718395 |
|  | CBS 121342; E.G.S. 42.122; IMI 310506 (A. pulcherrimae ${ }^{\mathrm{R}}$ ) | Australia, Euphorbia pulcherrima | KJ718223 | KJ718057 | KJ718731 | KJ718569 | KJ718396 |
|  | CBS 135189; E.G.S. 45.053 <br> (A. solani ${ }^{R}$ ) | New Zealand, Solanum tuberosum | KJ718224 | GQ180082 | GQ180098 | KJ718570 | KJ718397 |
| Alternaria pseudorostrata | CBS 119411; E.G.S. $42.060^{\text {T }}$ | USA, Euphorbia pulcherrima | JN383483 | AY562406 | AY563295 | KC584680 | KC584422 |
| Alternaria ranunculi | CBS 116330; E.G.S. 38.039; IMI 285697 | Israel, Ranunculus asiaticus | KJ718225 | KJ718058 | KJ718732 | KJ718571 | KJ718398 |
| Alternaria ricini | CBS $215.31^{\text {T }}$ | Japan, Ricinus communis | KJ718226 | KJ718059 | KJ718733 | KJ718572 | KJ718399 |
|  | CBS 353.86 | Italy, Ricinus communis | KJ718227 | JQ646331 | KJ718734 | KJ718573 | KJ718400 |
|  | CBS 117361; E.G.S. $06.181^{\text {R }}$ | USA, Ricinus communis | KJ718228 | KJ718060 | KJ718735 | KJ718574 | KJ718401 |
| Alternaria rostellata | CBS 117366; E.G.S. $42.061^{\text {T }}$ | USA, Euphorbia pulcherrima | KJ718229 | JQ646332 | KJ718736 | KJ718575 | KJ718402 |
| Alternaria scorzonerae | CBS 103.46; Elliot No. 45190C (A. linicola) | UK, Linum usitatissimum | KJ718190 | JQ646363 | JQ646447 | KJ718537 | KJ718363 |
|  | CBS 478.83; E.G.S. 38.011 ${ }^{\text {T }}$ | Netherlands, Scorzonera hispanica | KJ718191 | JQ646334 | KJ718699 | KJ718538 | KJ718364 |
|  | CBS 116703; E.G.S. 36.110; IMI 274549 (A. linicola ${ }^{\mathrm{R}}$ ) | UK, Linum usitatissimum | KJ718192 | KJ718031 | KJ718700 | KJ718539 | KJ718365 |
| Alternaria sennae sp. nov. | CBS 477.81; E.G.S. 34.030; <br> IMI $257253^{\mathrm{T}}$ (A. cassiae ${ }^{\mathrm{R}}$ ) | India, Senna corymbosa | KJ718230 | JQ646344 | JQ646428 | EU130543 | KJ718403 |
| Alternaria sesami | CBS 240.73 | Egypt, Sesamum indicum | KJ718231 | JQ646343 | KJ718737 | KJ718576 | KJ718404 |
|  | CBS 115264; CBS 117214; E.G.S. $13.027^{\mathrm{R}}$ | India, Sesamum indicum | JF780939 | KJ718061 | KJ718738 | KJ718577 | KJ718405 |
| Alternaria sidae | CBS 117730; E.G.S. $12.129^{\text {T }}$ | Kiribati, Sida fallax | KJ718232 | KJ718062 | KJ718739 | KJ718578 | KJ718406 |
| Alternaria silybi | CBS 134092; VKM F-4109 ${ }^{\text {T}}$ | Russia, Silybum marianum | KJ718233 | KJ718063 | KJ718740 | KJ718579 | KJ718407 |
|  | CBS 134093; VKM F-4117 | Russia, Silybum marianum | KJ718234 | KJ718064 | KJ718741 | KJ718580 | KJ718408 |



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| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | ITS | GAPDH | Alt a 1 | TEF1 | RPB2 |
| Alternaria thunbergiae | CBS 479.81; E.G.S. 33.081; GST 556² | UK, Tagetes erecta | KC584221 | KC584143 | KJ718761 | KC584692 | KC584434 |
|  | CBS 480.81; E.G.S. $33.184^{\text {R }}$ | USA, Tagetes sp. | KJ718255 | KJ718082 | KJ718762 | KJ718601 | KJ718430 |
|  | CBS 117217; E.G.S. 44.045 ${ }^{\text {R }}$ | USA, Tagetes sp. | KJ718256 | KJ718083 | KJ718763 | KJ718602 | KJ718431 |
|  | CBS 116331; E.G.S. 41.073; BRIP $14963{ }^{\text {T }}$ | Australia, Thunbergia alata | KJ718257 | KJ718084 | KJ718764 | KJ718603 | KJ718432 |
|  | CBS 120986; E.G.S. 51.075 <br> (A. iranica ${ }^{\mathrm{T}}$ ) | Iran, Allium сера | KJ718258 | KJ718085 | KJ718765 | KJ718604 | KJ718433 |
|  | CBS 122597 | New Zealand, Thunbergia alata | KJ718259 | KJ718086 | KJ718766 | KJ718605 | KJ718434 |
| Alternaria tillandsiae | CBS 116116; E.G.S. $43.074^{\text {T }}$ | New Zealand, Tillandsia usneoides | KJ718260 | KJ718087 | KJ718767 | KJ718606 | KJ718435 |
| Alternaria tropica | CBS 631.93; E.G.S. $39.126^{\text {T }}$ | USA, Passiflora edulis | KJ718261 | KJ718088 | KJ718768 | KJ718607 | KJ718436 |
|  | CBS 117216; E.G.S. $39.125^{\text {R }}$ | USA, Passiflora edulis | KJ718262 | KJ718089 | KJ718769 | KJ718608 | KJ718437 |
| Alternaria venezuelensis | CBS 116121; E.G.S. $48.065{ }^{\text {T }}$ | Venezuela, Phaseolus vulgaris | KJ718263 | KJ718090 | KJ718770 | KJ718609 | KJ718438 |
| Alternaria zinniae | CBS 118.44 | Hungary, Callistephus chinensis | KJ718264 | JQ646361 | KJ718771 | KJ718610 | KJ718439 |
|  | CBS 107.48 | Netherlands, Zinnia sp. | KJ718265 | KJ718091 | KJ718772 | KJ718611 | KJ718440 |
|  | CBS 117.59 | Italy, Zinnia elegans | KJ718266 | KJ718092 | KJ718773 | KJ718612 | KJ718441 |
|  | CBS 108.61 | Unknown, Zinnia elegans | KJ718267 | KJ718093 | KJ718774 | KJ718613 | KJ718442 |
|  | CBS 299.79 | UK, Zinnia sp. | KJ718268 | KJ718094 | KJ718775 | KJ718614 | KJ718443 |
|  | CBS 300.79 | UK, Zinnia sp. | KJ718269 | KJ718095 | KJ718776 | KJ718615 | KJ718444 |
|  | CBS 117223; E.G.S. $44.035^{\text {R }}$ | New Zealand, Zinnia elegans | KJ718270 | KJ718096 | KJ718777 | KJ718616 | KJ718445 | ${ }^{1}$ ATCC: American Type Culture Collection, Manassas, VA, USA; BRIP: Queensland Plant Pathology Herbarium, Queensland, Australia; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, Netherlands; CCM: Czech Collection of Microorganisms, Brno, Czech Republic; CECT: Spanish Type Culture Collection, Valencia, Spain; CPC: Personal collection of P.W. Crous, Utrecht, Netherlands; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; DSM: German Collection of Microorganisms and Cell Cultures, Leibniz Institute, Braunschweig, Germany; E.G.S.: Personal collection of Dr. E.G. Simmons; Elliott: Personal collection of M.E. Elliott; GST: Personal collection of G.S. Taylor; ICMP: International Collection of Micro-organisms from Plants, Auckland, New Zealand; IFO: Institute for Fermentation Culture Collection, Osaka, Japan; IMI: Culture collection of CABI Europe UK Centre, Egham UK; LEV: Plant Health and Diagnostic Station, Levin, New Zealand; MUCL: (Agro)Industrial Fungi and Yeast Collection of the Belgian Co-ordinated Collections of Micro-organisms (BCCM), Louvain-la Neuve, Belgium; Nattrass: Personal collection of R.M. Nattrass; PD: Plant Protection Service, Wageningen, Netherlands; PPRI: ARC-Plant Protection Research Institute, Roodeplaat, South Africa; QM: Quarter Master Culture Collection, Amherst, MA, USA; VKM: All-Russian Collection of Microorganisms,Moscow, Russia.

${ }^{2}$ T: ex-type strain; R: representative strain; Bold letters are designated in this study.; Species names between parentheses refer to the former species names.

## Sect. Porri



Fig. 1. Bayesian 50 \% majority rule consensus tree based on the ITS, GAPDH, RPB2, TEF1 and Alt a 1 sequences of 183 Alternaria strains. The Bayesian posterior probabilities $>0.75(\mathrm{PP})$ and RAxML bootstrap support values $>65(\mathrm{ML})$ are given at the nodes (PP/ML). Thickened lines indicate a PP of 1.0 and ML of 100 . Species names between parentheses represent synonymised species names. Ex-type strains are indicated with T and representative strains with R. Novel species names are printed in bold face. The tree was rooted to A. gypsophilae (CBS 107.41).


Fig. 1. (Continued).

Sect. Porri


Fig. 1. (Continued).


Fig. 2. Bayesian $50 \%$ majority rule consensus tree based on the GAPDH, RPB2 and TEF1 sequences of 41 Alternaria strains. The Bayesian posterior probabilities (PP) are given at the nodes. Thickened lines indicate a PP of 1.0. The tree was rooted to $A$. brassicicola (CBS 118699).

| Species name | Synonymised names (this study) | Host / Substrate |
| :---: | :---: | :---: |
| Alternaria acalyphicola |  | Euphorbiaceae (Acalypha indica) |
| Alternaria agerati |  | Asteraceae (Ageratum houstonianum) |
| Alternaria agripestis |  | Euphorbiaceae (Euphorbia esula) |
| Alternaria allii | Alternaria vanuatuensis | Amaryllidaceae (Allium cepa, A. porrum) |
| Alternaria alternariacida |  | Solanaceae (Solanum lycopersicum) |
| Alternaria anagallidis |  | Primulaceae (Anagallis arvensis) |
| Alternaria anodae |  | Malvaceae (Anoda cristata) |
| Alternaria aragakii |  | Passifloraceae (Passiflora edulis) |
| Alternaria argyroxiphii |  | Asteraceae (Argyroxiphium sp.), Convolvulaceae (Ipomoea batatas) |
| Alternaria azadirachtae |  | Meliaceae (Azadirachta indica) |
| Alternaria bataticola |  | Convolvulaceae (Ipomoea batatas) |
| Alternaria blumeae | Alternaria brasilliensis | Asteraceae (Blumea aurita), Fabaceae (Phaseolus vulgaris) |
| Alternaria calendulae | Alternaria rosifolii | Asteraceae (Calendula officinalis), <br> Rosaceae (Rosa sp.) |
| Alternaria carthami | Alternaria heliophytonis | Asteraceae (Carthamus tinctorius, Helianthus annuus) |
| Alternaria carthamicola |  | Asteraceae (Carthamus tinctorius) |
| Alternaria cassiae | Alternaria hibiscinficiens <br> Alternaria sauropodis | Fabaceae (Senna obtusifolia), Malvacea (Hibiscus sabdariffa), Phyllanthaceae (Sauropus androgynus) |
| Alternaria catananches |  | Asteraceae (Catananche caerulea) |
| Alternaria centaureae |  | Asteraceae (Centaurea solstitialis) |
| Alternaria cichorii |  | Asteraceae (Cichorium endivia, C. intybus) |
| Alternaria cirsinoxia |  | Asteraceae (Cirsium arvense) |
| Alternaria citrullicola |  | Cucurbitaceae (Citrullus lanatus) |
| Alternaria conidiophora |  | Unknown |
| Alternaria crassa | Alternaria capsici | Solanaceae (Capsicum annuum, Datura stramonium, Nicandra physalodes) |
| Alternaria cucumerina | Alternaria loofahae | Cucurbitaceae (Cucumis melo, Luffa acutangula) |
| Alternaria cyamopsidis |  | Fabaceae (Cyamopsis tetragonoloba) |
| Alternaria dauci | Alternaria poonensis | Apiaceae (Daucus carota, Coriandrum sativum), Asteraceae (Cichorium intybus) |
| Alternaria deserticola |  | Soil |
| Alternaria dichondrae |  | Convolvulaceae (Dichondra sp., D. repens) |
| Alternaria echinaceae |  | Asteraceae (Echinacea sp.) |
| Alternaria grandis |  | Solanaceae (Solanum tuberosum) |
| Alternaria ipomoeae |  | Convolvulaceae (Ipomoea batatas) |
| Alternaria jesenskae |  | Cistaceae (Fumana procumbens) |

Table 2. (Continued).

| Species name | Synonymised names (this study) | Host / Substrate |
| :---: | :---: | :---: |
| Alternaria linariae | Alternaria cretica <br> Alternaria cucumericola <br> Alternaria subcylindrica <br> Alternaria tabasco <br> Alternaria tomatophila | Cucurbitaceae (Cucumis sativus), Scrophulariaceae (Linaria maroccana), Solanaceae (Capsicum frutescens, Solanum lycopersicum) |
| Alternaria macrospora |  | Malvaceae (Gossypium sp., G. barbadense) |
| Alternaria montanica |  | Asteraceae (Cirsium arvense) |
| Alternaria multirostrata |  | Rubiaceae (Richardia scabra) |
| Alternaria neoipomoeae |  | Convolvulaceae (Ipomoea batatas) |
| Alternaria nitrimali |  | Solanacaea (Solanum viarum) |
| Alternaria novae-guineensis |  | Asteraceae (Galinsoga parviflora), Rutaceae (Citrus sp.) |
| Alternaria obtecta |  | Euphorbiaceae (Euphorbia pulcherrima) |
| Alternaria paralinicola |  | Linaceae (Linum usitatissimum) |
| Alternaria passiflorae | Alternaria gaurae <br> Alternaria hawaiiensis | Onagraceae (Gaura lindheimeri), Passifloraceae (Passiflora edulis, P. caerulea, P. ligularis), Solanaceae (Capsicum frutescens) |
| Alternaria pipionipisi |  | Euphorbiaceae (Euphorbia pulcherrima), Fabaceae (Cajanus cajan) |
| Alternaria porri |  | Amaryllidaceae (Allium cepa, A. porrum) |
| Alternaria protenta | Alternaria hordeiseminis <br> Alternaria pulcherrimae | Asteraceae (Helianthus annuus), Euphorbiaceae (Euphorbia pulcherrima), Gramineae (Hordeum vulgare), Solanaceae (Solanum lycopersicum, S. tuberosum) |
| Alternaria pseudorostrata |  | Euphorbiaceae (Euphorbia pulcherrima) |
| Alternaria ranunculi |  | Ranunculaceae (Ranunculus asiaticus) |
| Alternaria ricini |  | Euphorbiaceae (Ricinus communis) |
| Alternaria rostellata |  | Euphorbiaceae (Euphorbia pulcherrima) |
| Alternaria scorzonerae | Alternaria linicola | Asteraceae (Sorzonerae hispanica), Linaceae (Linum usitatissimum) |
| Alternaria sennae |  | Fabaceae (Senna corymbosa) |
| Alternaria sesami |  | Pedaliaceae (Sesamum indica) |
| Alternara sidae |  | Malvaceae (Sida fallax) |
| Alternaria silybi |  | Asteraceae (Silybum marianum) |
| Alternaria solani | Alternaria danida <br> Alternaria viciae-fabae | Asteraceae (Ageratum houstonianum), Fabaceae (Vicia faba), Solanaceae (Solanum aviculare, S. tuberosum) |

Table 2. (Continued).

| Species name | Synonymised names (this study) | Host / Substrate |
| :---: | :---: | :---: |
| Alternaria solani-nigri | Alternaria ascaloniae <br> Alternaria beticola <br> Alternaria cyphomandrae <br> Alternaria glyceriae <br> Alternaria herbiculinae | Amaryllidaceae (Allium ascalonicum), Apiaceae (Petroselinum crispum), Chenopodiaceae (Beta vulgaris), Gramineae (Glyceria maxima), Solanaceae (Cyphomandra betacea, Solanum nigrum) |
| Alternaria steviae |  | Asteraceae (Stevia rebaudiana) |
| Alternaria tagetica |  | Asteraceae (Tagetes sp., T. erecta) |
| Alternaria thunbergiae | Alternaria iranica | Acanthaceae (Thunbergia alata), Amaryllidaceae (Allium cepa) |
| Alternaria tillandsiae |  | Bromeliaceae (Tillandsia usneoides) |
| Alternaria tropica |  | Passifloraceae (Passiflora edulis) |
| Alternaria venezuelensis |  | Fabaceae (Phaseolus vulgaris) |
| Alternaria zinniae |  | Asteraceae (Callistephus chinensis, Zinnia sp., Z. elegans) |

## Taxonomy

At the onset of this study, Alternaria sect. Porri contained 82 Alternaria species. After extensive phylogenetic analyses and morphological examination we now recognise 63 species in this section (Table 2), of which 10 are newly described. Twenty-seven species names are reduced to synonymy (Table 2). All isolates where taxonomic changes were found based on the multigene phylogeny were studied morphologically; photo plates of these species are included. Type details are only listed when typification is proposed.

Section Porri D.P. Lawr., Gannibal, Peever \& B.M. Pryor, Mycologia 105: 541. 2013.
Type species: Alternaria porri (Ellis) Cif.
Section Porri is characterised by broadly ovoid, obclavate, ellipsoid, subcylindrical or obovoid, medium to large conidia, disto- and euseptate, solitary or in short chains, with a simple or branched, long to filamentous beak. Conidia contain multiple transverse and longitudinal septa and are slightly constricted near some transverse septa. Secondary conidiophores can be formed apically and / or laterally.

## Species in sect. Porri

Alternaria acalyphicola E.G. Simmons, Mycotaxon 50: 260. 1994.
Material examined: Seychelles, from Acalypha indica (Euphorbiaceae), before Apr. 1982, C. Kingsland, culture ex-type of A. acalyphicola CBS 541.94 = E.G.S. $38.100=$ IMI 266969.

Notes: Alternaria acalyphicola is closely related to A. ricini, with only 1 nt difference in three out of the five genes sequenced; RPB2, TEF1 and GAPDH. Based on this single isolate, the data is inconclusive to support the synonymy of these two species.

Alternaria agerati E.G. Simmons, Mycotaxon 65: 63. 1997.
= Alternaria agerati Sawada, Rep. Dept. Agric. Gov. Res. Inst. Formosa 86: 165. 1943. (nom. inval., Art. 36.1)

Material examined: USA, Illinois, Springfield, from Ageratum houstonianum (Asteraceae) in a commercial greenhouse, Nov. 1968, J.L. Forsberg, representative isolate of A. agerati CBS 117221 = E.G.S. 30.001 = QM 9369.

Alternaria agripestis E.G. Simmons \& K. Mort., Mycotaxon 50: 255. 1994.
Material examined: Canada, Saskatchewan, Maxim, from infected stem of Euphorbia esula (Euphorbiaceae), 9 Jul. 1992, P. Harris, culture ex-type of A. agripestis CBS 577.94 = E.G.S. 41.034.

Alternaria allii Nolla, Phytopathology 17: 118. 1927. Fig. 3.
$=$ Alternaria vanuatuensis E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 260. 2007.

Materials examined: Denmark, from seed of Allium cepa (Amaryllidaceae), 1937, P. Neergaard, CBS 109.41 = CBS 114.38. Italy, from leaf of Allium porrum (Amaryllidaceae), 1974, H. Nirenberg, CBS 225.76. Puerto Rico, from leaf of Allium cepa, before 1928, J.A.B. Nolla, culture ex-type of A. allii CBS 107.28 = E.G.S. 48.084. USA, Massachusetts, Hadley, from floral bract of Allium cepa var. viviparum, 13 Jul. 1980, E.G. Simmons, representative of A. allii CBS 116701 = E.G.S. 33.134. Vanuatu, from leaves of Allium cepa, 1996, C.F. Hill, culture ex-type of $A$. vanuatuensis CBS $121345=$ E.G.S 45.018 .

Notes: Simmons (2007) designated the lectotype of A. allii as Nolla (1927), loc. cit., Pl. III, fig. 11-19, based on the absence of original Nolla specimens. In our study, however, we managed to uncover an original specimen, CBS 107.28, which was deposited in the CBS by J.A.B. Nolla in December 1927 as his "A. allii sp. nov.", just after he published the new species description. We therefore recognise this isolate as the ex-type strain of $A$. allii. Isolate CBS 116701 did not sporulate after 3 wk of cultivation on SNA.

Alternaria alternariacida Woudenb. \& Crous, sp. nov. MycoBank MB808990. Fig. 4.
Etymology: Named after its ability to produce high amounts of alternaric acid.
Alternaria alternariacida differs from the ex-type isolate of its closest phylogenetic neighbour $A$. silybi (CBS 134092) based on alleles in three loci (positions derived from respective alignments of the separate loci deposited in TreeBASE): ITS position 386 (T), 497 (T), 498 (T); TEF1 position $3(\mathrm{~T}), 18(\mathrm{~T})$; Alt a 1 position $205(\mathrm{C}), 336(\mathrm{~T}), 339(\mathrm{~A}), 350(\mathrm{C}), 404(\mathrm{~T}), 408(\mathrm{G})$.

Sporulation is atypical. Primary conidiophores solitary, simple, straight to slightly curved, septate, pale brown with a subhyaline tip, (52-)73-93(-155) $\times(4-) 5-6(-7) \mu \mathrm{m}$, bearing a


Fig. 3. Alternaria allii: conidia and conidiophores. A-C. CBS 107.28. D-E. CBS 109.41. F-H. CBS 225.76. I-L. CBS 121345. Scale bars $=10 \mu \mathrm{~m}$.
single, darkened, apical conidiogenous locus. Conidia solitary or in unbranched chains of $2(-3)$ conidia, conidium body pale olive-brown, smooth-walled, narrowly ovoid, solitary, non-catenulate, and secondary conidia (33-)44-49(-56) $\times(5-) 7-8(-9) \mu \mathrm{m}$, with (3-)5-6(-8) transverse eusepta and no longitudinal septa; primary conidia in total (85-)99-111(-121)× $(6-) 7-8(-10) \mu \mathrm{m}$. The conidial body can be slightly constricted near the septa. The conidium body gradually tapers into mostly an aseptate, single, unbranched beak, but branched beaks do occur; apical and multiple lateral secondary conidiophores can also occur. Beaks (47-)129-257(-610) $\mu \mathrm{m}$ long, $c a .2 \mu \mathrm{~m}$ wide throughout their length. Sexual morph not observed.


Fig. 4. Alternaria alternariacida sp. nov. CBS 105.51: A-H. Conidia and conidiophores. Scale bars $=$ $10 \mu \mathrm{~m}$.

Culture characteristics: After 7 d cultures on SNA flat, fimbriate, white; aerial mycelium sparse, white, colonies reaching $25-30 \mathrm{~mm}$ diam; cultures on PCA flat, entire, olivaceous in the centre with three olivaceous concentric circles and a buff to white margin; aerial mycelium fine, felty, white, colonies reaching 50 mm diam; reverse with four olivaceous concentric circles.

Material examined: UK, England, from fruit of Solanum lycopersicum (Solanaceae), 1946, P.W. Brian (holotype CBS H-21734, culture ex-type CBS 105.51 = ATCC 11078 = IMI 46816 $=$ CECT $2997=$ IBPG $14=$ BRL408).

Note: The atypical sporulation of the single isolate of A. alternariacida, which is over 60 yr old, resulted in our decision to include sequence data in the species description.

Alternaria anagallidis A. Raabe, Hedwigia 78: 87. 1939.
Materials examined: Denmark, Copenhagen, from Anagallis arvensis (Primulaceae), before Mar. 1944, P. Neergaard, CBS 107.44. New Zealand, Auckland, Lynfield, from Anagallis arvensis, 4 May 1998, C.F. Hill, CBS 101004; Auckland, Lynfield, from Anagallis arvensis, 28 Jun. 1995, C.F. Hill, representative isolate of A. anagallidis CBS 117128 = E.G.S. 42.074; Auckland, from leaf spot of Anagallis arvensis, Jan. 2002, C.F. Hill, representative isolate of $A$. anagallidis CBS $117129=$ E.G.S. 50.091.

Notes: Isolate CBS 107.44 differs on 6 nt positions in its RPB2 sequence from the other three $A$. anagallidis isolates included in this study. Because CBS 107.44 still clusters closest to the other A. anagallidis isolates, and since these isolates, from a single host species, form a distinct clade from all other Alternaria spp., we retained the name A. anagallidis for this isolate.

Alternaria anodae E.G. Simmons, Mycotaxon 88: 198. 2003.
Material examined: South Africa, Gauteng Province, Pretoria, ARC-Roodeplaat VOPI, from leaves of Anoda cristata (Malvaceae), 12 Jan. 2012, A. Thompson, PPRI 12376.

Alternaria aragakii E.G. Simmons, Mycotaxon 46: 181. 1993.
Material examined: USA, Hawaii, from Passiflora edulis (Passifloraceae), before Oct. 1968, M. Aragaki, culture ex-type of A. aragakii CBS 594.93 = E.G.S. $29.016=$ QM 9046.

Alternaria argyroxiphii E.G. Simmons \& Aragaki, Mycotaxon 65: 40. 1997.
Materials examined: South Africa, Gauteng Province, Pretoria, ARC-Roodeplaat VOPI, from stem lesion of Ipomoea batatas (Convolvulaceae), 20 Apr. 2005, A. Thompson, PPRI 11848; Mpumalanga Province, Marble Hall, from stem and leaf lesion of Ipomoea batatas, 22 Nov. 2011, A. Thompson, PPRI 11971. USA, Hawaii, Maui, Haleakala, from Argyroxiphium sp. (Asteraceae), 1969, M. Aragaki, culture ex-type of A. argyroxiphii CBS 117222 = E.G.S. 35.122.

Note: The host range of A. argyroxiphii is not restricted to Argyroxiphium, but has been broadened with the inclusion of two isolates from Ipomoea batatas (Convolvulaceae).

Alternaria azadirachtae E.G. Simmons \& Alcorn, CBS Biodiversity Ser. (Utrecht) 6: 218. 2007.

Materials examined: Australia, Queensland, Tewantin, from Azadirachta indica (Meliaceae), 20 Jul. 1998, A. Bradley, culture ex-type of A. azadirachtae CBS 116444 = E.G.S. $46.195=$ BRIP 25386 (ss1); additional strain from the same source, CBS $116445=$ E.G.S. $46.196=$ BRIP25386 (ss2).

Alternaria bataticola W. Yamam., Trans. Mycol. Soc. Japan 2(5): 89. 1960.
= Macrosporium bataticola Ikata, Agric. Hort. (Tokyo) 22: 241. 1947 (nom. inval., Art. 36.1).

Type: (Lectotype, designated in Simmons 2007) S. Ikata, Agric. \& Hort. 22: 241. fig. 1. 1947.
Materials examined: Australia, Queensland, Walkamin, from leaf spot of Ipomoea batatas (Convolvulaceae), 5 Jul. 1991, collector unknown, representative isolate of $A$. bataticola CBS 117095 = E.G.S. $42.157=$ IMI $350492=$ BRIP 19470a; additional strain from the same source CBS 117096 = E.G.S. 42.158 = BRIP 19470b. Japan, Tokyo, from Ipomoea batatas, before Nov. 1963, collector unknown, CBS 532.63; from Ipomoea batatas, before Nov. 1963, collector unknown (epitype designated here CBS H-21743, MBT178114, culture ex-epitype


Fig. 5. Alternaria blumeae: conidia and conidiophores. A-D. CBS 117364. E-H. CBS 117215. Scale bars $=10 \mu \mathrm{~m}$.

CBS 531.63 = IFO 6187 = MUCL 28916). South Africa, Gauteng Province, Pretoria, ARCRoodeplaat VOPI, from leaf and stem lesion of Ipomoea batatas, 16 Jun. 2010, M. Truter, PPRI 10502; Kwazulu-Natal Province, Empangeni, from leaf lesion of Ipomoea batatas, 4 Jul. 2011, A. Thompson, PPRI 11930; Kwazulu-Natal Province, Empangeni, from leaf lesion of Ipomoea batatas, 4 Jul. 2011, A. Thompson, PPRI 11931; Gauteng Province, Pretoria, ARC-Roodeplaat VOPI, from leaf lesion of Ipomoea batatas, 12 Jan. 2012, A. Thompson, PPRI 11934.

Alternaria blumeae E.G. Simmons \& Sontirat, Mycotaxon 65: 81. 1997. Fig. 5.
= Alternaria brasiliensis F.M. Queiroz, M.F.S. Muniz \& M. Menezes, Mycopathologia 150: 63. 2001.

Materials examined: Brazil, Espirito Santo, from leaf spot of Phaseolus vulgaris (Fabaceae), 1989, F.M. Queiroz, representative isolate of $A$. brasiliensis CBS 117215 = E.G.S. 39.116. Thailand, Yala Province, Amphoe Muang, from Blumea aurita (Asteraceae), 18 Jan. 1992, P. Sontirat, culture ex-type of A. blumeae CBS $117364=$ E.G.S. $40.149=$ ATCC 201357.

Notes: By synonymising $A$. brasiliensis with $A$. blumeae, the host range of this taxon has expanded to include Phaseolus vulgaris. The five sequenced genes are $100 \%$ identical between the two examined specimens.


Fig. 6. Alternaria calendulae: conidia and conidiophores. A-C. CBS 224.76. D-E. CBS 101498. F-H. CBS 116650. I-L. CBS 116439. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria calendulae Ondřej, Čas. Slez. Mus., Ser. A, Hist. Nat. 23: 150. 1974. Fig. 6.
= Alternaria calendulae W. Yamam. 1939 (nom. nud.).
$=$ Macrosporium calendulae Nelen, Bull. Centr. Bot. Gard. (Moscow) 35: 90. 1959 (nom. inval., Art. 36.1).
= Macrosporium calendulae Nelen, Bot. Mater. Otd. Sporov. Rast. Bot. Inst. Akad. Nauk S.S.S.R. 15: 144. 1962.
= Alternaria calendulae Nirenberg, Phytopathol. Z. 88: 108. 1977 (nom. illegit., Art. 53.1).
$=$ Alternaria rosifolii E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 192. 2007.


Fig. 7. Alternaria carthami: conidia and conidiophores. A-D. CBS 117091. E-H. CBS 116440. Scale bars $=10 \mu \mathrm{~m}$.

Materials examined: Germany, former West-Germany, from leaf spot of Calendula officinalis (Asteraceae), 1974, H. Nirenberg, culture ex-type of A. calendulae Nirenberg CBS 224.76 $=$ ATCC 38903 = IMI $205077=$ DSM 63161. Japan, Tokyo, from leaf spot of Calendula officinalis, before 1964, representative isolate of A. calendulae CBS $116650=$ E.G.S. $30.142=$ QM 9561. New Zealand, Auckland, Kumeu, from leaf spot of Calendula officinalis, Oct. 1998, C.F. Hill, CBS 101498; Auckland, Mount Albert, from leaf of Rosa sp. (Rosaceae), before Feb. 1995, C.F. Hill, culture ex-type of $A$. rosifolii CBS 116439 = E.G.S. 42.197 .

Note: By synonymising A. rosifolii with A. calendulae, the host range of this taxon has expanded to include Rosa.

Alternaria carthami S. Chowdhury, J. Indian Bot. Soc. 23: 65. 1944. Fig. 7.
= Macrosporium anatolicum A. Săvul., Bull. Sect. Sci. Acad. Roumaine 26: 709. 1944.
= Alternaria heliophytonis E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 206. 2007.
Materials examined: Canada, Saskatchewan, Saskatoon, from leaf of Helianthus annuus (Asteraceae), 26 Aug. 1993, C. Jasalavich, culture ex-type of A. heliophytonis CBS $116440=$ IMI 366164 = E.G.S. 43.143. Italy, Perugia, from leaf of Carthamus tinctorius (Asteraceae), before Nov. 1980, A. Zazzerini, CBS 635.80. USA, Montana, Sidney, from leaf spot of Carthamus tinctorius, 11 Jul. 1973, E.E. Burns, representative isolate of A. carthami CBS 117091 = E.G.S. 31.037.


Fig. 8. Alternaria carthamicola sp. nov. CBS 117092: A-L. Conidia and conidiophores. Scale bars $=$ $10 \mu \mathrm{~m}$.

Notes: Isolate CBS 635.80 did not sporulate after 3 wk cultivation on SNA. By synonymising $A$. heliophytonis with $A$. carthami, the host range of this taxon has expanded to include Helianthus annuus (Asteraceae).

Alternaria carthamicola Woudenb. \& Crous, sp. nov. MycoBank MB808991. Fig. 8.
Etymology: Named after the host genus from which it was collected, Carthamus.
Primary conidiophores solitary or in small groups, simple, straight to slightly curved, septate, pale to dark brown with a subhyaline tip, (33-)55-71(-108) $\times 5-6(-7) \mu \mathrm{m}$, bearing a single,
darkened, apical conidiogenous locus, but may produce geniculate conidiogenous extensions. Conidia solitary, rarely in chains of two conidia, conidium body pale olive-brown, mostly smooth-walled but sometimes ornamented at the base, ovoid, (39-)58-64(-82) $\times(13-) 15-$ $16(-17) \mu \mathrm{m}$; with $(5-) 6-7(-9)$ transverse and (1-)3(-4) longitudinal septa. Dark coloured eusepta can be formed during development; the conidial body is slightly constricted near the transverse septa. Conidia mostly have a septate, single to double filamentous beak, triple beaks are observed but not common, apical secondary conidiophores can be formed. Beaks (40-) 158-186(-219) $\mu \mathrm{m}$ long, $c a .2 \mu \mathrm{~m}$ diam throughout their length and $4 \mu \mathrm{~m}$ at the base. Sexual morph not observed.

Culture characteristics: After 7 d cultures on SNA flat, rhizoid, white to opaque; aerial mycelium sparse, white, floccose, colonies reaching $55-60 \mathrm{~mm}$ diam; cultures on PCA flat, entire, olivaceous with three clear concentric circles; aerial mycelium fine, felty, olivaceous to olivaceous-grey, colonies reaching $65-70 \mathrm{~mm}$ diam; reverse shows four olivaceous concentric circles with an buff edge.

Material examined: Iraq, from Carthamus tinctorius (Asteraceae), 10 Apr. 1983, M.M. Elsahookie (holotype CBS H-21735, culture ex-type CBS 117092 = IMI 276943 = E.G.S. 37.057).

Notes: The new species $A$. carthamicola, originally identified as $A$. carthami, differs only on 9 nt positions in its RPB2 sequence from the other two $A$. carthami strains studied. Based on its RPB2 sequence it clusters with $A$. linicola.

Alternaria cassiae Jurair \& A. Khan, Pakistan J. Sci. Industr. Res. 3: 72. 1960. Fig. 9.
= Alternaria hibiscinficiens E.G. Simmons \& C.F. Hill, Mycotaxon 88: 205. 2003.
$=$ Alternaria sauropodis E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 340. 2007.
Materials examined: Brazil, Federal District, from leaf spot of Senna obtusifolia (Fabaceae), May 1990, G. Fiqueiredo, representative isolate of $A$. cassiae CBS 117224 = E.G.S. 40.121. Fiji, from leaf of Hibiscus sabdariffa (Malvaceae), Jun. 2002, C.F. Hill, culture ex-type of $A$. hibiscinficiens CBS 177369 = E.G.S. 50.166. Malaysia, Sarawak, Kuching, from Sauropus androgynus (Phyllanthaceae), 25 Apr. 1984, T.K. Kieh, culture ex-type of A. sauropodis CBS 116119 = IMI 286317 = IMI 392448 = E.G.S. 47.112. USA, Mississippi, Stoneville, from diseased seedling of Senna obtusifolia, before Oct. 1980, H.L. Walker, representative isolate of A. cassiae CBS $478.81=$ E.G.S. 33.147.

Notes: Isolate CBS 478.81 did not sporulate after 3 wk incubation on SNA. By synonymising A. hibiscinficiens and $A$. sauropodis with $A$. cassiae, the host range of this taxon has expanded to include Sauropus androgynus (Euphorbiaceae) and Hibiscus sabdariffa (Malvaceae).

Alternaria catananches Woudenb. \& Crous, sp. nov. MycoBank MB808992. Fig. 10.
Etymology: Named after its host genus from which it was isolated, Catananche.
Primary conidiophores solitary, simple, straight to curved, septate, pale brown, (31-)54-67($94) \times(5-) 6(-7) \mu \mathrm{m}$, bearing a single, darkened, apical conidiogenous locus, but may produce


Fig. 9. Alternaria cassiae: conidia and conidiophores. A-D. CBS 116119. E-H. CBS 117224. I-L. CBS 117369. Scale bars $=10 \mu \mathrm{~m}$.
geniculate conidiogenous extensions. Conidia solitary, conidium body pale olive-brown, ornamented in lower half of the conidium, narrowly ovoid, (26-)37-43(-57) $\times(7-) 8-9(-11)$ $\mu \mathrm{m}$, with (2-)4(-6) transverse septa and no longitudinal septa. Some darker coloured eusepta can be formed during development. The conidium body gradually tapers into a single, septate, unbranched beak; basal lateral secondary conidiophores can be formed. Beaks (77-)126-160(260) $\mu \mathrm{m}$ long, $c a .2 \mu \mathrm{~m}$ diam throughout their length. Sexual morph not observed.

Culture characteristics: After 7 d cultures on SNA flat, entire / fimbriate, olivaceous around agar plug, white; aerial mycelium felty, white to olivaceous, colonies reaching $10-15 \mathrm{~mm}$


Fig. 10. Alternaria catananches sp. nov. A-B. Disease symptoms on Catananche caerulea (photo's K.H. Nugteren, Florensis B.V., Netherlands). C-L. CBS 137456: conidia and conidiophores. Scale bars $=$ $10 \mu \mathrm{~m}$.
diam; cultures on PCA flat, erose, grey-olivaceous; aerial mycelium fine felty, olivaceous-grey; colonies reaching 25 mm diam; reverse identical.

Material examined: Netherlands, from Catananche caerulea (Asteraceae), 11 Dec. 2013, N. Troost-Riksen (holotype CBS H-21736, culture ex-type CBS $137456=$ PD 013/05703936).

Notes: Alternaria catananches seems closely related to the $A$. cichorii isolates in the multi-gene phylogeny, but this is probably caused by long-branch attraction and incongruency between the different gene trees. Based on the ITS sequence it is identical to $A$. jesenskae, with RPB2 it is
identical to A. cirsinoxia, with TEF1 it clusters with A. cichorii / A. cirsinoxia / A. carthami and with Alt a 1 it is identical to $A$. cichorii CBS 102.33, A. alternariacida and $A$. scorzonerae. Only its GAPDH sequences make it distinct from all other Alternaria species. Although the multigene tree does not provide strong support for separating it from the $A$. cichorii isolates, based on the individual gene sequences it is described here as a new Alternaria species.

Alternaria centaureae E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 236. 2007.
Material examined: USA, California, Sacramento, from Centaurea solstitialis (Asteraceae), Feb. 1999, D. Fogle, culture ex-type of A. centaureae CBS 116446 = E.G.S. 47.119.

Alternaria cichorii Nattrass, First List of Cyprus Fungi: 29. 1937.
$\equiv$ Alternaria porri f. sp. cichorii (Nattrass) T. Schmidt, Pflanzenschutzberichte 32: 181. 1965.
$\equiv$ Macrosporium cichorii (Nattrass) Gordenko, Mikol. Fitopatol. 9: 241. 1975.
Materials examined: Cyprus, from leaf spot of Cichorium intybus (Asteraceae), 1933, R.M. Nattrass (holotype IMI 1007, culture ex-type CBS $102.33=$ E.G.S. $07.017=$ QM 1760). Greece, Attica, from Cichorium endivia (Asteraceae), 24 Feb. 1978, S.D. Demetriades, representative isolate of $A$. cichorii CBS $117218=$ E.G.S. $52.046=$ IMI 225641.

Notes: Strain CBS 102.33 was deposited in Aug. 1933 in the CBS by R.M. Nattrass as $A$. cichorii sp. nov., with the remark that the description of the new species was in preparation. The holotype was subsequently deposited in IMI (IMI 1007) which consists of a dried herbarium specimen. In the present study we link CBS 102.33 as ex-type of A. cichorii to IMI 1007. The two isolates used in this study, CBS 102.33 and CBS 117218, differ only on 7 nt positions in their Alt a 1 sequence. Unfortunately CBS 102.33 is sterile, which does not provide additional information to support them as being two different species. Furthermore, the time difference of 45 yr between isolation of the two strains led to the decision to retain them as one species for now, pending fresh collections.

Alternaria cirsinoxia E.G. Simmons \& K. Mort., Mycotaxon 65: 72. 1997.
Material examined: Canada, Saskatchewan, Watrous, from stem lesion and top dieback of Cirsium arvense (Asteraceae), 5 Aug. 1993, K. Mortensen, culture ex-type of A. cirsinoxia CBS 113261 = E.G.S. 41.136.

Alternaria citrullicola Woudenb. \& Crous, sp. nov. MycoBank MB808993. Fig. 11.
Etymology: Named after the host genus from which it was collected, Citrullus.
Primary conidiophores solitary, simple, straight or sometimes curved, septate, pale brown with a subhyaline tip, (28-)35-52(-73) $\times(3-) 4(-5) \mu \mathrm{m}$, bearing a single, darkened, apical conidiogenous locus. Conidia mostly solitary but chains of two conidia can occur, conidium body pale olive-brown, smooth-walled, narrowly ovoid, (28-)35-41(-56) $\times(6-) 8(-10) \mu \mathrm{m}$; with (3-)5-6(-9) transverse distosepta and $0-1(-2)$ longitudinal septa. Conidia have a single, aseptate, unbranched filamentous beak; apical secondary conidiophores can be formed. Beaks


Fig. 11. Alternaria citrullicola sp. nov. CBS 103.32: A-H. Conidia and conidiophores. Scale bars $=10$ $\mu \mathrm{m}$.
(72-)178-232(-324) $\mu \mathrm{m}$ long, ca. $2 \mu \mathrm{~m}$ diam throughout their length. Sexual morph not observed.

Culture characteristics: After 7 d cultures on SNA flat, fimbriate, white to opaque with primrose sections near the edge; aerial mycelium sparse, fine felty, colonies reaching $45-50 \mathrm{~mm}$ diam; cultures on PCA flat, entire, olivaceous with three unclear concentric circles; aerial mycelium is sparse, pale olivaceous-grey, colonies reaching $50-55 \mathrm{~mm}$ diam; reverse shows olivaceous-buff to olivaceous rings.

Material examined: Cyprus, from fruit of Citrullus lanatus (Cucurbitaceae), before Jul. 1932, R.M. Nattrass (holotype CBS H-21742, culture ex-type CBS $103.32=$ VKM F-1881).

Alternaria conidiophora Woudenb. \& Crous, sp. nov. MycoBank MB808995. Fig. 12.
Etymology: Named after its characteristically long, thick, conidiophores.
Primary conidiophores solitary, simple, mostly straight but sometimes curved, septate, dark brown with a subhyaline tip, (46-)89-105(-152) $\times(6-) 7(-8) \mu \mathrm{m}$, bearing a single to multiple, darkened, long geniculate conidiogenous loci. Conidia solitary, conidium body olive-brown, smooth-walled, narrowly ovoid, (30-)45-52(-66) $\times(10-) 12-13(-18) \mu \mathrm{m}$, with (2-)6-7(-9)


Fig. 12. Alternaria conidiophora sp. nov. CBS 137457: A-H. Conidia and conidiophores. Scale bars = $10 \mu \mathrm{~m}$.
transverse septa and (0-)1-2(-4) longitudinal septa. Darker coloured eusepta are formed during development. The conidial body is slightly constricted near the transverse septa. Conidia have a single, septate, unbranched, filamentous beak; basal, lateral secondary conidiophores can be formed. Beaks (49-)117-138(-186) $\mu \mathrm{m}$ long; ca. $2 \mu \mathrm{~m}$ diam throughout their length. Sexual morph not observed.

Culture characteristics: After 7 d cultures on SNA flat, fimbriate to rhizoid, white to opaque; aerial mycelium felty, white, colonies reaching $55-60 \mathrm{~mm}$ diam; cultures on PCA flat, entire, grey-olivaceous with two concentric circles; aerial mycelium wooly, pale olivaceous-grey, colonies reaching $55-60 \mathrm{~mm}$ diam; reverse identical.

Material examined: Netherlands, from unidentified host, Jul. 2011, U. Damm (holotype CBS H-21737, culture ex-type CBS 137457).

Alternaria crassa (Sacc.) Rands, Phytopathology 7: 337. 1917. Fig. 13.
Basionym: Cercospora crassa Sacc., Michelia 1(no. 1): 88. 1877.
$=$ Macrosporium solani Cooke, Grevillea 12: 32. 1883. (non M. solani Ellis \& Martin, 1882)
= Cercospora daturae Peck, Rep. New York State Mus. Nat. Hist. 35: 140. 1884.
= Macrosporium cookei Sacc., Syll. Fungorum 4: 530. 1886. (nom. nov. in Saccardo for M. solani Cooke, 1883, non M. solani Ellis \& Martin, 1882)


Fig. 13. Alternaria crassa: conidia and conidiophores. A-D. CBS 109162. E-H. CBS 116648. I-L. CBS 119160. Scale bars $=10 \mu \mathrm{~m}$.
$\equiv$ Alternaria cookei (Sacc.) Bremer, Ismen, Karel, Özkan \& M. Özkan, Istanbul Üniv. Fak. Mecm., B. 13: 42. 1948.
$=$ Macrosporium daturae Fautrey, Rev. Mycol. (Toulouse) 16: 76. 1894.
$\equiv$ Alternaria daturae (Fautrey) Bubák \& Ranoj., Fungi Imperf. Exsicc. Fasc. 14: 694. 1911.
= Alternaria capsici E.G. Simmons, Mycotaxon 75: 84. 2000.
Type: (Lectotype, designated in Simmons 2000) PAD, Cercospora crassa, Datura stramonium, S. [elva] '76. 10.

Materials examined: Australia, from Capsicum annuum (Solanaceae), May 1981, D. Trimboli, culture ex-type of $A$. capsici CBS $109160=$ IMI $262408=$ IMI $381021=$ E.G.S 45.075. Cyprus, Famagusta, from leaves of Datura stramonium (Solanaceae), Jan. 1936, R.M. Nattrass (epitype designated here CBS H-21744, MBT178115, culture ex-epitype CBS 110.38). New Zealand, Auckland, from leaf spot of Datura stramonium, 2002, C.F. Hill, representative isolate of $A$. crassa CBS $116448=$ E.G.S. 50.180. USA, Indiana, Montgomery County, Nicandra physalodes (Solanaceae), 5 Sep. 1997, E.G. Simmons, CBS 109162 = E.G.S. 46.014; Indiana, from leaf spot of Datura stramonium, 5 Sep. 1997, E.G. Simmons, representative isolate of $A$. crassa CBS 116447 = E.G.S. 46.013; Indiana, Montgomery County, from leaf spot of Datura stramonium, 1 Aug. 1996, E.G. Simmons, representative isolate of $A$. crassa CBS $122590=$ E.G.S. 44.071 ; Wisconsin, Madison, from leaf spot of Datura sp., before Apr. 1918, R.D. Rands, CBS 103.18.

Notes: Isolates CBS 110.38 and CBS 116647 did not sporulate after 3 wk incubation on SNA. By synonymising $A$. capsici with $A$. crassa, the host range of this taxon expanded to include Capsicum annuum, which also belongs to the Solanaceae.

Alternaria cucumerina (Ellis \& Everh.) J.A. Elliott, Amer. J. Bot. 4: 472. 1917. Fig. 14.
Basionym: Macrosporium cucumerinum Ellis \& Everh., Proc. Acad. Nat. Sci. Philadelphia 47: 440. 1895.
= Alternaria loofahae E.G. Simmons \& Aragaki, CBS Biodiversity Ser. (Utrecht) 6: 316. 2007.

Materials examined: Australia, Queensland, from leaf spot of Cucumis melo (Cucurbitaceae), Oct. 1996, R. O'Brien, representative isolate of A. cucumerina CBS 117226 = E.G.S. $44.197=$ BRIP 23060. USA, Hawaii, Oahu, Waialua, from Luffa acutangula (Cucurbitaceae), 1971, M. Aragaki, culture ex-type of $A$. loofahae CBS 116114 = E.G.S. 35.123; Indiana, Knox County, from leaf spot of Cucumis melo, 1993, R.X. Latin, representative isolate of A. cucumerina CBS 117225 = E.G.S. 41.127.

Notes: The species clade for A. cucumerina does not have a clear support in the multi-gene phylogeny. CBS 117225 and CBS 117226 differ only on 2 nt in their RPB2 sequence, while the ex-type of $A$. loofahae (CBS 116114) differs on 1 nt from both $A$. cucumerina isolates in RPB2 and on 1 nt in Alt a 1. This internal variation in the two $A$. cucumerina isolates and the identical host family, Cucurbitaceae, with A. loofahae, supported the synonymy of A. loofahae. By synonymising $A$. loofahae with $A$. cucumerina, the host range of this taxon expanded to include Luffa acutangula.

Alternaria cyamopsidis Rangaswami \& A.V. Rao, Indian Phytopathol. 10: 23. 1957.
$\equiv$ Alternaria cucumerina var. cyamopsidis (Rangaswami \& A.V. Rao) E.G. Simmons, Mycopathol. Mycol. Appl. 29: 131. 1966.

Materials examined: USA, Georgia, from leaf spot of Cyamopsis tetragonoloba (Fabaceae), Jul. 1961, G. Sowell, representative isolate of $A$. cyamopsidis CBS 117219 = E.G.S. 13.120 = QM 8000; Maryland, Beltsville, from leaf spot of Cyamopsis tetragonoloba, 1964, R.G. Orellana, representative isolate of A. cyamopsidis CBS $364.67=$ E.G.S. $17.065=$ QM 8575.


Fig. 14. Alternaria cucumerina: conidia and conidiophores. A-D. CBS 117225. E-H. CBS 117226. I-L. CBS 116114. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria dauci (J.G. Kühn) J.W. Groves \& Skolko, Canad. J. Res., Sect. C, Bot. Sci. 22: 222. 1944. Fig. 15.

Basionym: Sporidesmium exitiosum var. dauci J.G. Kühn, Hedwigia 1: 91. 1855.
$\equiv$ Polydesmus exitiosus var. dauci (J.G. Kühn) J.G. Kühn, Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung: 165. 1858.
$\equiv$ Macrosporium dauci (J.G. Kühn) Rostr., Tidsskr. Landoekon. ser. 5, 7: 385. 1888.
$\equiv$ Alternaria brassicae var. dauci (J.G. Kühn) Lindau, Rabenhorst's Kryptog.-Fl., Edn 2 (Leipzig) 1(9): 260. 1908.
$\equiv$ Alternaria porri $f$. sp. dauci (J.G. Kühn) Neerg, Danish species of Alternaria \& Stemphylium: 252. 1945.


Fig. 15. Alternaria dauci. A. Disease symptoms on Daucus carota. B-L. Conidia and conidiophores. B-C. CBS 117097. D-F. CBS 117098. G-I. CBS 117099. J-L. CBS 117100. Scale bars $=10 \mu \mathrm{~m}$.
$=$ Macrosporium carotae Ellis \& Langl., J. Mycol. 6: 36. 1890.
$\equiv$ Alternaria carotae (Ellis \& Langl.) J.A. Stev. \& Wellman, J. Wash. Acad. Sci. 34: 263. 1944.
= Alternaria poonensis Ragunath, Mycopathol. Mycol. Appl. 21: 315. 1963.
Type: (Lectotype, designated in Simmons 1995) B, ms. spec. Sporidesmium exitiosum var. dauci Kühn, Leg. Gross Krausche p. Bunzlau, Jul. Kühn.

Materials examined: Italy, from seed of Daucus carota (Apiaceae), Sept. 1937, P. Neergaard (neotype designated here CBS H-21745, MBT178116, culture ex-neotype CBS 111.38).

Netherlands, Limburg, Horst, from leaf spot in Cichorium intybus var. foliosum (Asteraceae), 1979, W.M. Loerakker, CBS 477.83 = CBS 721.79 = PD 79/954; from seed of Daucus carota, 1993, S\&G Seeds, CBS 101592. New Zealand, from leaf spot of Daucus carota, Mar. 1998, C.F. Hill, representative isolate of $A$. dauci CBS $117098=$ E.G.S. 46.152; Ohakune, from leaf spot of Daucus carota, before Jul. 1979, G.F. Laundon, CBS $345.79=$ LEV 14814. Puerto Rico, from seedling of Coriandrum sativum (Apiaceae), 1999, W. Almodovar, representative isolate of A. poonensis CBS $117100=$ E.G.S. 47.138 . Unknown, from seed of Daucus carota, Jan. 1948, J.W. Groves, CBS 106.48. USA, California, from commercial seed of Daucus carota, Nov. 1994, B.M. Pryor, representative isolate of $A$. dauci CBS 117097 = E.G.S. 46.006; California, Kern County, from seed of Daucus carota, 1999, D. Fogle, representative isolate of A. dauci CBS $117099=$ E.G.S. 47.131.

Notes: The indicated lectotype cannot be traced in B, and appears to be lost. We therefore designate CBS 111.38 as neotype. The isolates CBS 111.38, CBS 345.79 and CBS 101592 did not sporulate after 3 wk incubation on SNA.

Alternaria deserticola Woudenb. \& Crous, sp. nov. MycoBank MB808996.
Etymology: Named after the substrate from which it was isolated, namely desert soil.
Culture sterile.
Alternaria deserticola differs from the ex-type strain of its closest phylogenetic neighbour $A$. thunbergiae (CBS 116331) based on alleles in all five loci (positions derived from respective alignments of the separate loci deposited in TreeBASE): ITS position 165 (-), 373 (T), 381 (C), 383 (C), 488 (A); GAPDH position $484(\mathrm{~T})$; RPB2 position $76(\mathrm{C}), 88(\mathrm{~T}), 91(\mathrm{~T}), 139(\mathrm{C}), 211$ (T), 316 (T), 490 (C), 496 (A), 646 (T), 670 (C), 671 (T), 673 (A), 760 (G); TEF1 position 37 (C), 49 (G), 197 (A), 223 (A), 274 (T), 277(-), 311(T); Alt a 1 position 10 (C), 209 (A), 210 (T), 220 (G), 322 (T), 452 (G).

Culture characteristics: After 7 d cultures on SNA flat, rhizoid, olivaceous-buff; aerial mycelium absent, colonies reaching 55 mm diam; cultures on PCA flat, entire, five grey-olivaceous concentric circles; aerial mycelium sparse, colonies reaching 75-80 mm diam; reverse shows five olivaceous-grey rings.

Material examined: Namibia, from desert soil, 2001, M. Christensen (holotype CBS H-21738, culture ex-type CBS 110799).

Note: The clear phylogenetic distinction of the sterile culture of $A$. deserticola from all other strains included in this study, resulted in our decision to describe this species based on sequence data only.

Alternaria dichondrae Gambogi, Vannacci \& Triolo, Trans. Brit. Mycol. Soc. 65(2): 323. 1975.
Materials examined: Italy, Pisa, from leaf spot of Dichondra repens (Convolvulaceae), Mar. 1974, P. Gambogi, ex-isotype of A. dichondrae CBS 199.74 = E.G.S. 38.007; Pisa, from leaf spot of Dichondra repens, Mar. 1974, P. Gambogi, living lectotype of A. dichondrae


Fig. 16. Alternaria grandis: conidia and conidiophores. A-D. CBS 109158. E-H. CBS 116695. Scale bars $=10 \mu \mathrm{~m}$.

CBS 200.74 = E.G.S. 38.008. New Zealand, from leaf spot of Dichondra repens, before 1979, G.F. Laundon, CBS 346.79; Auckland, Lynfield, from leaf of Dichondra sp., Apr. 1991, C.F. Hill, representative isolate of $A$. dichondrae CBS $117127=$ E.G.S. 40.057.

Note: Simmons (2007) designated a lectotype with ex-lectotype strain (CBS 200.74), as he found the ex-isotype strain (CBS 199.74) to be sterile.

Alternaria echinaceae E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 318. 2007.

Materials examined: New Zealand, Gisborne, Makaraka, from leaf of Echinacea sp. (Asteraceae), Jan. 1998, C.F. Hill, culture ex-type of A. echinaceae CBS 116117 = E.G.S. 46.081; Gisborne, Makaraka, from leaf of Echinacea sp., Jan. 1998, C.F. Hill, representative isolate of A. echinaceae CBS 116118 = E.G.S. 46.082.

Alternaria grandis E.G. Simmons, Mycotaxon 75: 96. 2000. Fig. 16.
Materials examined: USA, Pennsylvania, Centre County, from leaf lesion of Solanum tuberosum (Solanaceae), Sep. 1966, B.J. Christ, culture ex-type of A. grandis CBS 109158 = E.G.S. 44.106; Pennsylvania, Clarion County, from leaf spot of Solanum tuberosum, Sep. 1966, B.J. Christ, representative isolate of $A$. grandis CBS $116695=$ E.G.S 44.108.

Notes: Although $A$. grandis differs by only 1 nt in its GAPDH sequence from $A$. solani, we retain it as a distinct species. Conidia of $A$. grandis are substantially larger than those of $A$. solani, and a recently published study could separate A. solani (CBS 109157) and A. grandis (CBS 109158) based on partial calmodulin gene sequence data (Gannibal et al. 2014).

Alternaria ipomoeae M. Truter, Woudenb. \& Crous, sp. nov. MycoBank MB808997. Fig 17.
Etymology: Named after the host genus on which it occurs, Ipomoea.
Primary conidiophores simple to branched, straight to slightly curved, septate, pale brown, (10-)51-73(-145) $\times(4-) 5 \mu \mathrm{~m}$, bearing a single to multiple, darkened, geniculate conidiogenous loci. Conidia mostly solitary but chains of two conidia can occur, conidium body olive-brown, smooth-walled with ornamented base, long ellipsoid to obclavate, (53-)60-65(-76) $\times(9-) 12(-$ 15) $\mu \mathrm{m}$, with ( $6-) 8-9(-12)$ transverse septa and $(0-) 2(-3)$ longitudinal septa. Up to four dark coloured eusepta can be formed during development; the conidial body is constricted near these eusepta. Conidia have a septate, single to double, filamentous beak; apical and lateral secondary conidiophores can be formed. Beaks (47-)136-162(-221) $\mu \mathrm{m}$ long, single beaks generally longer than multiple beaks, $c a .2 \mu \mathrm{~m}$ diam throughout their length, and approx. $3 \mu \mathrm{~m}$ diam at the base. Sexual morph not observed.

Culture characteristics: After 7 d cultures on SNA are flat, fimbriate, white; aerial mycelium sparse, felty, white, colonies reaching 50 mm diam; cultures on PCA flat, entire, grey-olivaceous with some darker sections; aerial mycelium fine felty, pale olivaceous-grey, colonies reaching $65-70 \mathrm{~mm}$ diam; reverse identical.

Materials examined: Ethiopia, from black lesions of Ipomoea batatas (Convolvulaceae), Jun. 1978, A.H.C. van Bruggen (holotype CBS H-21739, culture ex-type CBS 219.79). South Africa, Gauteng Province, Pretoria, ARC-Roodeplaat VOPI, from stem lesions of Ipomoea batatas, 16 Nov. 2006, C.D. Narayanin (paratype PREM 60979, culture ex-paratype PPRI 8988).

Alternaria jesenskae Labuda, P. Eliáš \& Sterfl., Microbiol. Res. 163: 209. 2008.
Material examined: Slovakia, district of the village Muzla, Podunajská nizina lowland, from seeds of Fumana procumbens (Cistaceae), Aug. 1999, P. Eliás jr., culture ex-type of A. jesenskae CBS $133855=$ CCM 8361.

Alternaria linariae (Neerg.) E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 677. 2007. Fig. 18.

Basionym: Alternaria anagallidis var. linariae Neerg., Danish species of Alternaria \& Stemphylium: 297. 1945.
= Alternaria cretica E.G. Simmons \& Vakal., Mycotaxon 75: 64. 2000.
= Alternaria subcylindrica E.G. Simmons \& R.G. Roberts, Mycotaxon 75: 62. 2000.
= Alternaria tomatophila E.G. Simmons, Mycotaxon 75: 53. 2000.
$=$ Alternaria cucumericola E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 210. 2007.
$=$ Alternaria tabasco E.G. Simmons \& R.G. Roberts, CBS Biodiversity Ser. (Utrecht) 6: 158. 2007.


Fig. 17. Alternaria ipomoeae sp. nov. CBS 219.79: A-L. Conidia and conidiophores. Scale bars $=10$ $\mu \mathrm{m}$.

Materials examined: Belgium, host unknown, before Mar. 1961, R. Sys, CBS 107.61. Denmark, from seedling of Linaria maroccana (Scrophulariaceae), 13 Nov. 1940, P. Neergaard, culture ex-type of A. linariae CBS $105.41=$ E.G.S. 07.016. Greece, Crete, Heraklio, from leaf spot of Solanum lycopersicum (Solanaceae), 1997, D.J. Vakalounakis, culture ex-type of A. cretica, CBS 109164 = E.G.S. 46.188. New Zealand, Northland, Kerikeri, from leaf spot of Cucumis sativus (Cucurbitaceae), Mar. 1993, C.F. Hill, culture ex-type of A. cucumericola CBS 116438 = E.G.S.41.057. Thailand, Chiang Mai, Royal project, from leaf spot of Solanum lycopersicum, 5 Nov. 2012, P.W. Crous, CPC 21620 . Unknown, host unknown, before Apr. 1953, P.W. Brian, CBS 108.53 = No. 408P. USA, Indiana, Montgomery County, from leaf spot of Solanum


Fig. 18. Alternaria linariae. A. Disease symptoms on Solanum lycopersicum. B-P. Conidia and conidiophores. B-C. CBS 105.41. D-F. CBS 109161. G-H. CBS 107.61. I-J. CBS 109156. K-L. CBS 109164. M-N. CBS 116438. O-P. CBS 116441. Scale bars $=10 \mu \mathrm{~m}$.
lycopersicum, 23 Aug. 1995, E.G. Simmons, culture ex-type of A. tomatophila CBS $109156=$ E.G.S. 42.156; Indiana, from leaf lesion of Solanum lycopersicum, Aug. 1996, E.G. Simmons, representative isolate of $A$. tomatophila CBS $116704=$ E.G.S. 44.074; Louisiana, Baton Rouge, Louisiana State University Burden Research Plantation, from leaf lesion of Solanum lycopersicum var. cerasiforme, 2 Jul. 1997, R.G. Roberts, culture ex-type of $A$. subcylindrica CBS 109161 = E.G.S. 45.113; Louisiana, Avery Island, from leaf spot of Capsicum frutescens (Solanaceae), 1 Jul. 1997, R.G. Roberts, culture ex-type of A. tabasco CBS 116441 = E.G.S 45.108 = R.G.R. 97-52.

Notes: By synonymising A. cretica, A. cucumericola, A. subcylindrica, A. tabasco and A. tomatophila with A. linariae, the broad host range of this taxon now consists of Solanaceae, Cucurbitaceae and Scrophulariaceae species. The isolates CBS 108.53 and CBS 116704 did not sporulate on SNA after 3 wk of incubation.

Alternaria macrospora Zimm., Ber. Land-Forstw. Deutsch-Ostafrika 2: 24. 1904. $\equiv$ Macrosporium macrosporum (Zimm.) Nishikado \& Oshima, Agric. Res. (Kurashiki) 36: 391. 1944.
= Sporidesmium longipedicellatum Reichert, Bot. Jahrb. Syst. 56: 723. 1921.
$\equiv$ Alternaria longipedicellata (Reichert) Snowden, Rep. Dept. Agric. Uganda: 31. 1927 [1926].

Materials examined: Nigeria, from Gossypium sp. (Malvaceae), May 1929, Jones, CBS 106.29. USA, Arizona, from Gossypium barbadense (Malvaceae), before 1984, P.J. Cotty, culture epitype of $A$. macrospora CBS $117228=$ E.G.S. $50.190=$ ATCC 58172.

Notes: Isolate CBS 106.29 was preserved in the CBS collection as A. porri, but did not sporulate since 1978. Based on our molecular data this isolate belongs to $A$. macrospora, which, based on the same host, seems plausible.

Alternaria montanica E.G. Simmons \& Robeson, CBS Biodiversity Ser. (Utrecht) 6: 178. 2007.
Material examined: USA, Montana, from Cirsium arvense (Asteraceae), before Apr. 1981, D.J. Robeson, culture ex-type of $A$. montanica CBS $121343=$ E.G.S. $44.112=$ IMI 257563.

Alternaria multirostrata E.G. Simmons \& C.R. Jacks., Phytopathology 58: 1139. 1968.
Materials examined: USA, Georgia, Tifton, from floral bract of Richardia scabra (Rubiaceae), 1967, C.R. Jackson, culture ex-type of $A$. multirostrata CBS $712.68=$ ATCC $18515=$ IMI $135454=$ MUCL $11722=$ QM $8820=$ VKM-F2997; Georgia, Tifton, from floral bract of Richardia scabra, 1967, C.R. Jackson, representative isolate of $A$. multirostrata CBS $713.68=$ ATCC $18517=$ IMI $135455=$ MUCL $11715=$ QM 8821 .

Alternaria neoipomoeae M. Truter, Woudenb. \& Crous, sp. nov. MycoBank MB808998. Fig. 19.

Etymology: Named after its close phylogenetic relationship to $A$. ipomoeae.


Fig. 19. Alternaria neoipomoeae sp. nov. A. Disease symptoms on Ipomoeae batatas (Photo A.H. Thompson, ARC, South Africa). B-L. PPRI 11845: conidia and conidiophores. Scale bars $=10 \mu \mathrm{~m}$.

Primary conidiophores solitary, simple, straight to slightly curved, septate, pale brown, (10-) $23-59(-111) \times(4-) 5 \mu \mathrm{~m}$, bearing a single, darkened, apical conidiogenous locus, which may produce 1-2 geniculate conidiogenous extensions. Conidia are mostly solitary but chains of two conidia can occur, conidium body olive-brown, smooth-walled with ornamented base, long ellipsoid to obclavate, (52-)66-77(-93) $\times(12-) 14-16(-18) \mu \mathrm{m}$, with (7-)9(12) transverse and (2-)3-4(-5) longitudinal septa. Up to four dark coloured eusepta can be formed during development; the conidial body is constricted near these eusepta. Conidia mostly have a septate, single to double, filamentous beak, triple beaks are observed but not common; apical and lateral secondary conidiophores can be formed. Beaks (54-)104-136(-
200) $\mu \mathrm{m}$ long, $c a .2 \mu \mathrm{~m}$ diam throughout their length, and approx. $3 \mu \mathrm{~m}$ diam at the base. Sexual morph not observed.

Culture characteristics: After 7 d cultures on SNA flat, fimbriate, white to opaque; aerial mycelium sparse, fine felty, white, colonies reaching $60-65 \mathrm{~mm}$ diam; cultures on PCA flat, entire, grey-olivaceous with 2 dark and one lighter concentric circles and a pale olivaceous edge; aerial mycelium fine felty, pale olivaceous-grey, colonies reaching $55-60 \mathrm{~mm}$ diam; reverse four olivaceous-grey rings.

Materials examined: South Africa, Gauteng Province, Pretoria, ARC-Roodeplaat VOPI, from stem lesion of Ipomoea batatas (Convolvulaceae), 8 Jun. 2011, A. Thompson (holotype PREM 60981, culture ex-type PPRI 11845); North-West Province, Brits, from Ipomoea batatas, 25 Oct. 2007, C.D. Narayanin (paratype PREM 60982, culture ex-paratype PPRI 8990); Mpumalanga Province, Kwamahlanga, from Ipomoea batatas, between 2006 and 2008, C.D. Narayanin (paratype PREM 60983, culture ex-paratype PPRI 11847); Gauteng Province, Pretoria, ARC-Roodeplaat VOPI, from leaf lesion of Ipomoea batatas, Oct. 2013, A. Thompson (paratype PREM 60984, culture ex-paratype PPRI 13903).

Alternaria nitrimali E.G. Simmons \& M.E. Palm, Mycotaxon 75: 93. 2000.
Material examined: Puerto Rico, Luquillo, from leaf spot of Solanum viarum (Solanaceae), 26 Feb. 1998, USDA-APHIS, culture ex-type of A. nitrimali CBS 109163 = E.G.S 46.151.

Alternaria novae-guineensis E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 350. 2007.

Materials examined: Papua New Guinea, from dried leaf of Citrus sp. (Rutaceae) imported to New Zealand, 1999, C.F. Hill, culture ex-type of A. novae-guineensis CBS 116120 = E.G.S. 47.198. South Africa, Gauteng, Pretoria, ARC-Roodeplaat VOPI, from leaves of Galinsoga parviflora (Asteraceae), 12 Jan. 2012, A. Thompson, PPRI 12171.

Alternaria obtecta E.G. Simmons, Mycotaxon 50: 250. 1994.
Materials examined: USA, California, Encinitas, from leaf of Euphorbia pulcherrima (Euphorbiaceae), Nov. 1994, C.F. Hill, representative isolate of A. obtecta CBS 117367 = E.G.S. 42.063; California, Encinitas, from Euphorbia pulcherrima (Euphorbiaceae), Nov. 1994, C.F. Hill, CBS 134278 = E.G.S. 42.064.

Alternaria paralinicola Woudenb. \& Crous, sp. nov. MycoBank MB808999. Fig. 20.
Etymology: Named after its close phylogenetic relationship to A. linicola.
Primary conidiophores solitary, simple, straight to slightly curved, septate, pale brown, (39-) $64-82(-133) \times(4-) 5-6 \mu \mathrm{~m}$, bearing a single, darkened, apical conidiogenous locus, but may produce geniculate conidiogenous extensions. Conidia are mostly solitary but chains of two conidia can occur, conidium body pale olive-brown, smooth-walled, narrowly ovoid, (31-)39-$44(-58) \times(8-) 10-11(-15) \mu \mathrm{m}$, with $(3-) 5-6(-8)$ transverse septa and $0-1(-2)$ longitudinal


Fig. 20. Alternaria paralinicola sp. nov. CBS 116652: A-L. Conidia and conidiophores. Scale bars $=$ $10 \mu \mathrm{~m}$.
septa. Dark coloured eusepta are formed during maturation. The conidial body is slightly constricted near the transverse septa. Some transverse blocks of cells can have a conspicuously different width in comparison with neighbouring segments, resulting in specific shape of the conidium body. Conidia mostly have a single, aseptate, unbranched, filamentous beak; double beaks are observed but not common; apical or lateral secondary conidiophores can be formed. Beaks (61-)114-135(-169) $\mu \mathrm{m}$ long, ca. $2 \mu \mathrm{~m}$ diam throughout their length. Sexual morph not observed.

Culture characteristics: After 7 d cultures on SNA flat, fimbriate, white to opaque; aerial mycelium sparse, white, colonies reaching $70-75 \mathrm{~mm}$ diam; cultures on PCA flat, entire,
grey-olivaceous with four olivaceous clear concentric circles; aerial mycelium is fine felty, olivaceous, colonies reaching 70 mm diam; reverse shows five grey-olivaceous concentric circles.

Material examined: Canada, Manitoba, from seeds of cultivated Linum usitatissimum (Linaceae), 1996, M.E. Corlett (holotype CBS H-21740, culture ex-type CBS 116652 = E.G.S. 47.157 = DAOM 225747).

Note: Alternaria paralinicola, which was originally identified as A. linicola, differs on 16 nt positions in its RPB2 sequence from the other two $A$. linicola strains studied. Based on its RPB2 sequence it clusters with $A$. passiflorae.

Alternaria passiflorae J.H. Simmonds, Proc. Roy. Soc. Queensland. 49: 151. 1938. Fig. 21.
= Alternaria hawaiiensis E.G. Simmons, Mycotaxon 46: 184. 1993.
$=$ Alternaria gaurae E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 188. 2007.

Materials examined: New Zealand, from fruit of Passiflora edulis (Passifloraceae), 6 Feb. 1963, F.J. Mortin, representative isolate of $A$. passiflorae CBS $629.93=$ E.G.S. $16.150=$ QM 8458; Auckland, from fruit spot of Passiflora ligularis (Passifloraceae), Apr. 2004, C.F. Hill, representative isolate of $A$. passiflorae CBS $117102=$ E.G.S. 51.165; Auckland, from leaf spot of Passiflora caerulea (Passifloraceae), Jul. 2004, C.F. Hill, representative isolate of A. passiflorae CBS 117103 = E.G.S. 52.032; Auckland, from leaf spot of Gaura lindheimeri (Onagraceae), May 2002, C.F. Hill, culture ex-type of A. gaurae CBS 116333 = E.G.S. 50.121; Waitakere, from leaf of Capsicum frutescens (Solanaceae), May 1975, CBS 166.77. USA, Hawaii, from Passiflora edulis, before Oct. 1968, M. Aragaki, culture ex-type of A. hawaiiensis CBS 630.93 = E.G.S. $29.020=$ QM 9050.

Notes: By synonymising A. gaurae with A. passiflorae, and including CBS 166.77, formerly identified as $A$. solani, the host range of $A$. passiflorae has broadened to include Gaura sp. (Onagraceae) and Capsicum frutescens (Solanaceae).

Alternaria pipionipisi E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 302. 2007.
Materials examined: India, Andhra Pradesh, Hyderabad, from seed of Cajanus cajan (Fabaceae), before Feb. 1990, K.M. \& Ch. Reddy, culture ex-type of A. pipionipisi CBS 116115 = E.G.S. $40.096=$ IMI 340950. USA, California, Encinitas, from Euphorbia pulcherrima (Euphorbiaceae), Sep. 1994, C.F. Hill, CBS 134265 = E.G.S. 42.047; California, Encinitas, from Euphorbia pulcherrima, Sep. 1994, C.F. Hill, representative isolate of A. obtecta CBS 117365 = E.G.S. 42.048.

Alternaria porri (Ellis) Cif., J. Dept. Agric. Porto Rico 14: 30. 1930 [1929]. Fig. 22.
Basionym: Macrosporium porri Ellis, Grevillea 8 (no. 45): 12. 1879.
$\equiv$ Alternaria porri (Ellis) Sawada, Rep. Dept. Agric. Gov. Res. Inst. Formosa, 61: 92. 1930.


Fig. 21. Alternaria passiflorae: conidia and conidiophores. A-B. CBS 117102. C-D. CBS 117103. E-F. CBS 116333. G-H. CBS 166.77. I-J. CBS 630.93. K-L. CBS 629.93. Scale bars $=10 \mu \mathrm{~m}$.

Type: (Lectotype, designated in Simmons 2007) NY, Ellis Collection: on leaves of Allium porrum, Newfield, N.J. Sept. 78.

Materials examined: USA, Nebraska, Lincoln, from leaf of Allium cepa (Amaryllidaceae), 1965, D.S. Meredith, representative isolate of A. allii CBS $116649=$ E.G.S. $17.082=$ QM 8613; New York, Ithaca, from leaf of Allium cepa, 1996, M.J. Yáñes Morales, representative isolate of A. porri CBS 116698 = E.G.S. 48.147; New York, Orange County, from leaf of Allium cepa, 1996, M.J. Yáñes Morales (epitype designated here CBS H-21746, MBT178117, culture exepitype CBS 116699 = E.G.S. 48.152).

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Fig. 22. Alternaria porri: conidia and conidiophores. A-D. CBS 116698. E-H. CBS 116699. I-L. CBS 116649. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria protenta E.G. Simmons, Mycotaxon 25: 207. 1986. Fig. 23.
= Alternaria pulcherrimae T.Y. Zhang \& J.C. David, Mycosystema 8-9: 110. 1996.
$=$ Alternaria hordeiseminis E.G. Simmons \& G.F. Laundon, CBS Biodiversity Ser. (Utrecht) 6: 150. 2007.

Materials examined: Australia, Queensland, Brisbane, Chapel Hill, from Euphorbia pulcherrimae (Euphorbiaceae), 25 Aug. 1986, J.L. Alcorn, representative isolate of A. pulcherrimae CBS 121342 = E.G.S. $42.122=$ IMI 310506. Israel, from Helianthus annuus (Asteraceae), 1996, collector unknown, representative isolate of A. protenta CBS $116697=$ E.G.S. $45.024=$ IMI 372957; from Helianthus annuus, 1996, collector unknown, representative isolate of A. protenta CBS 116696


Fig. 23. Alternaria protenta: conidia and conidiophores. A-B. CBS 116696. C-D. CBS 116697. E-G. CBS 116643. H-J. CBS 116651. K-M. CBS 121342. N-P. CBS 347.79. Scale bars $=10 \mu \mathrm{~m}$.
= E.G.S. 45.023 = IMI 372955. New Zealand, Hastings, from Solanum tuberosum (Solanaceae), Mar. 1997, C.F. Hill, representative isolate of A. solani CBS 135189 = E.G.S. 45.053; Levin, from fruit rot of Solanum lycopersicum (Solanaceae), before Jul. 1979, G.F. Laundon, CBS 347.79 = E.G.S. $44.091=$ ATCC $38569=$ LEV 14726; Palmerston North, from seed of Hordeum vulgare (Gramineae), Jul. 1977, G.F. Laundon, culture ex-type of A. hordeiseminis CBS 116437 = E.G.S. $32.076=$ CBS 116443 = E.G.S. 46.163. USA, California, Siskiyou, from Solanum tuberosum, 1996, D. Fogle, representative isolate of $A$. solani CBS 116651 = E.G.S. 45.020.

Notes: By synonymising $A$. pulcherrimae and $A$. hordeiseminis with $A$. protenta and including three isolates formerly identified as $A$. solani (CBS 347.79, 116651 and 135189), the host range of $A$. protenta has expanded extensively. It now comprises plants from the Asteraceae, Euphorbiaceae, Gramineae and Solanaceae. Based on molecular (and morphological) data, A. protenta is closely related to $A$. solani, and these two species can only be distinguished based on 9 nt differences in their RPB2 sequences (see RPB2 alignment in TreeBASE).

Alternaria pseudorostrata E.G. Simmons, Mycotaxon 57: 398. 1996.
Material examined: USA, California, Encinitas, from Euphorbia pulcherrimae (Euphorbiaceae), Dec. 1994, C.F. Hill, culture ex-type of $A$. pseudorostrata CBS 119411 = E.G.S. 42.060.

Alternaria ranunculi E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 212. 2007.
Material examined: Israel, Palestine, from seed of Ranunculus asiaticus (Ranunculaceae), 10 Apr. 1984, collector unknown, culture ex-type of A. ranunculi CBS $116330=$ E.G.S. $38.039=$ IMI 285697.

Alternaria ricini (Yoshii) Hansf., Proc. Linn. Soc. Lond. : 53. 1943.
Basionym: Macrosporium ricini Yoshii, Bult. Sci. Fak. Terk. Kjusu Imp. Univ. 3(4): 327. 1929.
Type: (Lectotype, designated in Simmons 1994) BPI 445446, Macrosporium ricini, Japan, Fukuoka, Ricinus communis, July 1928.

Materials examined: Italy, Sardinia, Sasseri, from Ricinus communis (Euphorbiaceae), before Aug. 1986, J.A. von Arx, CBS 353.86. Japan, Ricinus communis, deposited Feb. 1931 by K. Nakata (epitype designated here CBS H-21747, MBT178118, culture ex-epitype CBS 215.31). USA, Virginia, Holland, from leaf of Ricinus communis, 9 Aug. 1954, C.A. Thomas, representative isolate of $A$. ricini $\mathrm{CBS} 117361=$ E.G.S. 06.181.

Alternaria rostellata E.G. Simmons, Mycotaxon 57: 401. 1996.
Material examined: USA, California, Encinitas, from leaf of Euphorbia pulcherrimae (Euphorbiaceae), Jan. 1995, C.F. Hill, culture ex-type of A. rostellata CBS $117366=$ E.G.S. 42.061.

Alternaria scorzonerae (Aderh.) Loer., Netherlands J. Pl. Pathol. 90(1): 37. 1984.
Basionym: Sporidesmium scorzonerae Aderh., Arbeiten Kaiserl. Biol. Anst. Land-Forstw. 3: 439. 1903.
= Alternaria linicola J.W. Groves \& Skolko, Canad. J. Res., Sect. C, Bot. Sci. 22: 223. 1944. = Alternaria linicola Neerg, Danish species of Alternaria \& Stemphylium: 302. 1945. (nom. illegit., Art. 53.1)

Type: (Lectotype, designated in Simmons 1997) Aderhold, Arbeiten Kaiserl. Biol. Anst. LandForstw. 3: 440. fig. w/o number. 1903.

Materials examined: Netherlands, Reusel, from leaf spot of Scorzonera hispanica (Asteraceae), 1982, W.M. Loerakker (epitype designated here CBS H-21748, MBT178119, culture exepitype CBS 478.83 = E.G.S. 38.011). UK, Scotland, from Linum usitatissimum (Linaceae), 22 Nov. 1945, J.W. Groves, CBS 103.46; Derbyshire, from seed of Linum usitatissimum, 1983, C. Nicholls, representative isolate of A. linicola CBS $116703=$ E.G.S. $36.110=$ IMI 274549.

Notes: None of the three isolates sporulated on SNA or PCA after 3 wk of incubation, also not after scarification. Corlett \& Corlett (1999) already stated that, after sub-cultivation, A. linicola sporulates poorly, or not at all. By synonymizing $A$. linicola with $A$. scorzonerae, the host range of $A$. scorzonerae is expanded to include Linum usitatissimum (Linaceae).

Alternaria sennae Woudenb. \& Crous, sp. nov. MycoBank MB809000. Fig. 24.
Etymology: Named after the host genus on which it occurs, Senna.
Primary conidiophores solitary, simple, straight to slightly curved, septate, dark brown with a hyaline tip, (43-)67-81(-108) $\times(5-) 6(-7) \mu \mathrm{m}$, bearing a single, darkened, apical conidiogenous locus, but may produce geniculate conidiogenous extensions. Conidia solitary, conidium body pale olive-brown, smooth-walled, narrowly ovoid, (46-)55-62(-69) $\times(8-) 10-12(-14) \mu \mathrm{m}$, with (7-)7-8(-10) transverse distosepta and (1-)2-3(-4) longitudinal septa. The conidial body can be slightly constricted near some transverse septa. Conidia have a single, aseptate, filamentous beak, which occasionally branches once; basal lateral secondary conidiophores can be formed. Beaks (38-)99-163(-314) $\mu \mathrm{m}$ long, ca. $2 \mu \mathrm{~m}$ diam. Sexual morph not observed.

Culture characteristics: After 7 d cultures on SNA flat, fimbriate, white to opaque with two olivaceous concentric circles; aerial mycelium sparse, white, floccose, colonies reaching 35-40 mm diam; cultures on PCA flat, undulate, white with grey-olivaceous zones; aerial mycelium felty, pale olivaceous-grey, colonies reaching $50-55 \mathrm{~mm}$ diam; reverse with pale olivaceousgrey zones.

Material examined: India, Uttar Pradesh, Gorakhpur, from leaf of Senna corymbosa (Fabaceae), 10 Apr. 1981, R.P. Verma (holotype CBS H-21741, culture ex-type CBS 477.81 = E.G.S. $34.030=$ IMI 257253).

Alternaria sesami (E. Kawam.) Mohanty \& Behera, Curr. Sci. 27: 493. 1958.
Basionym: Macrosporium sesami E. Kawam., Fungi 1: 27. 1931.
Materials examined: Egypt, from Sesamum indicum (Pedaliaceae), 1972, S.B. Mathur, CBS 240.73. India, from seedlings of Sesamum indicum, Dec. 1959, E.E. Leppik, representative isolate CBS $115264=$ CBS $117214=$ E.G.S. 13.027.


Fig. 24. Alternaria sennae sp. nov. CBS 477.81: A-L. Conidia and conidiophores. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria sidae E.G. Simmons, Mycotaxon 88: 202. 2003.
Material examined: Kiribati, Phoenix islands, Canton Island, from leaf spot of Sida fallax (Malvaceae), 11 Feb. 1958, O. \& I. Degener, culture ex-type of A. sidae CBS 117730 = E.G.S. 12.129.

Alternaria silybi Gannibal, Mycotaxon 114: 110. 2011.
Materials examined: Russia, Vladivostok, Trudovoe, from leaf lesion of Silybum marianum (Asteraceae), 1 Sep. 2006, Ph. B. Gannibal, culture ex-type of A. silybi CBS $134092=$


Fig. 25. Alternaria solani. A. Disease symptoms on Solanum tuberosum (Photo J.E. van der Waals, University of Pretoria, South Africa). B-H. Conidia and conidiophores. B-D. CBS 109157. E-H. CBS 116442. Scale bars $=10 \mu \mathrm{~m}$.

VKM F-4109; Vladivostok, Trudovoe, from leaf lesion of Silybum marianum, 1 Sep. 2006, Ph. B. Gannibal, CBS 134094 = VKM F-4118; Vladivostok, Botanical Garden-Institute, from leaf lesion of Silybum marianum, 6 Sep. 2006, Ph. B. Gannibal, CBS $134093=$ VKM F-4117.

Alternaria solani Sorauer, Z. Pflanzenkrankh. Pflanzenschutz 6: 6. 1896. Fig. 25.
$=$ Macrosporium solani Ellis \& G. Martin, Amer. Naturalist 16(12): 1003. 1882 (non M. solani Cooke, 1883)
$\equiv$ Alternaria solani (Ellis \& G. Martin) L.R. Jones \& Grout, Vermont Agric. Exp. Sta. Annual Rep. 9: 86. 1899. (nom. illegit., Art. 53.1)
$\equiv$ Alternaria americana Sawada, Rep. Dept. Agric. Gov. Res. Inst. Formosa 51:117. 1931. (nom. nov. for A. solani (Ellis \& G. Martin) L.R. Jones \& Grout (1899), non $A$. solani Sorauer (1896))
$\equiv$ Alternaria porri f. sp. solani (Ellis \& G. Martin) Neerg, Danish species of Alternaria \& Stemphylium: 260. 1945.
$=$ Sporidesmium solani-varians Vañha, Naturwiss. Z. Forst- Landw. 2: 117. 1904.
= Alternaria danida E.G. Simmons, Mycotaxon 65: 78. 1997.
$=$ Alternaria viciae-fabae E.G. Simmons \& G.F. Laundon, CBS Biodiversity Ser. (Utrecht) 6: 234. 2007.

Materials examined: Italy, from seed of Ageratum houstonianum (Asteraceae), 27 Aug. 1941, P. Neergaard, culture ex-type of $A$. danida CBS $111.44=$ E.G.S. $07.029=$ QM 1772. New Zealand, from Vicia faba (Fabaceae), Jun. 1979, G.F. Laundon, culture ex-type of $A$. viciaefabae CBS 116442 = E.G.S. 46.162 = ICMP 10242. Unknown, from leaf spot of Solanum aviculare (Solanaceae), before May 1941, P. Neergaard, CBS 111.41; unknown host, before Nov. 1921, isolated by Künkel, CBS 106.21. USA, Washington, Douglas County, from leaf spot of Solanum tuberosum (Solanaceae), 25 Aug. 1996, E.G. Simmons, representative isolate of $A$. solani CBS 109157= E.G.S. 44.098.

Notes: By synonymising A. danida and A. viciae-fabae with A. solani, the host range of this pathogen has expanded to include Asteraceae and Fabaceae host plants. The isolates CBS 106.21 and CBS 111.44 did not sporulate after 3 wk of incubation on SNA (both were already labelled as sterile in the CBS collection database). Isolate CBS 111.41 did sporulate, but the spore formation was atypical.

Alternaria solani-nigri R. Dubey, S.K. Singh \& Kamal [as "solani-nigrii"], Microbiol. Res. 154: 120. 1999. Fig. 26.
= Alternaria cyphomandrae E.G. Simmons, Mycotaxon 75: 86. 2000.
$=$ Alternaria ascaloniae E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 168. 2007.
$=$ Alternaria beticola E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 170. 2007.
= Alternaria glyceriae E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 148. 2007.
= Alternaria herbiculinae E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 166. 2007.

Materials examined: New Zealand, Canterbury, Ashburton, from leaf lesion of Beta vulgaris (Chenopodiaceae), Jul. 1999, B. Alexander, culture ex-type of A. beticola CBS 116447 = E.G.S. 47.196; Hastings, from leaf spot of Allium ascalonicum (Amaryllidaceae), Oct. 1997, C.F. Hill, culture ex-type of $A$. ascaloniae CBS $121347=$ E.G.S 46.052; New Plymouth, from fruit of Cyphomandra betacea (Solanaceae), May 1991, C.F. Hill, culture ex-type of A. cyphomandrae CBS 109155 = E.G.S. 40.058; Taranaki, Otaki, from stunted Petroselinum crispum (Apiaceae), 14 Jun. 2001, J.B. Wong, culture ex-type of $A$. herbiculinae CBS 116332 = E.G.S. 49.180; Waikato, Kopuku, from leaf spot of Glyceria maxima (Gramineae), Apr. 2003, C.F. Hill, culture ex-type of A. glyceriae CBS 116334 = E.G.S. 51.107; Waikato, Whangamarino swamp, from leaf spot of Solanum nigrum (Solanaceae), 21 Jun. 2003, C.F. Hill, representative isolate of A. solani-nigri CBS 113403 = E.G.S. $51.106=$ CPC 10620; Waikato, Whangamarino swamp, from leaf spot of Solanum nigrum, 6 Feb. 2003, C.F. Hill, representative isolate of A. solaninigri CBS 117101 = E.G.S. 51.032.

Notes: By synonymising these five Alternaria species with A. solani-nigri, this becomes a species with a broad host range found on Amaryllidaceae, Apiaceae, Chenopodiaceae, Gramineae and Solanaceae. All studied specimens originate from New Zealand, but the holotype of A. solaninigri was described from India. The five sequenced genes are $100 \%$ identical between all the specimens studied.


Fig. 26. Alternaria solani-nigri: conidia and conidiophores. A-B. CBS 113403. C-D. CBS 116447. E-G. CBS 109155. H-I. CBS 116334. J-K. CBS 121347. L-M. CBS 116332. N-P. CBS 117101. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria steviae Ishiba, T. Yokoy. \& Tani, Ann. Phytopathol. Soc. Japan 48(1): 46. 1982.
Materials examined: Japan, Kagawa, Kida-gun, Miki-cho, Ikenobe, from leaf spot of Stevia rebaudiana (Asteraceae), CBS $631.88=$ IFO 31212; Kagawa, Kida-gun, Miki-cho, Ikenobe, from leaf spot of Stevia rebaudiana, Jun. 1980, CBS $632.88=$ IFO 31183; Kagawa, Zentsuji, Harada-cho, from leaf spot of Stevia rebaudiana, Aug. 1978, C. Ishiba, culture ex-type of $A$. steviae CBS $117362=$ IFO $31182=$ E.G.S. 37.019.

Alternaria tagetica S.K. Shome \& Mustafee, Curr. Sci. 35: 370. 1966.
Materials examined: UK, from seed of Tagetes sp. (Asteraceae), before May 1979, G.S. Taylor, CBS 297.79; from seed of Tagetes sp., before May 1979, G.S. Taylor, CBS 298.79; England, Manchester, from seed of Tagetes erecta (Asteraceae), before Apr. 1980, G.S. Taylor, representative isolate of $A$. tagetica CBS 479.81 = E.G.S. 33.081 . USA, Ohio, Butler County, Oxford, from leaf of cultivated Tagetes sp., 14 Jun. 1996, M.A. Vincent, representative isolate of A. tagetica CBS 117217 = E.G.S 44.045 ; South Carolina, Clemson, from seed of Tagetes sp., before Mar. 1981, E. Smallwood Hotchkiss, representative isolate of A. tagetica CBS 480.81 = E.G.S. 33.184.

Alternaria thunbergiae E.G. Simmons \& Alcorn, CBS Biodiversity Ser. (Utrecht) 6: 136. 2007. Fig. 27.
$=$ Alternaria iranica E.G. Simmons \& Ghosta, CBS Biodiversity Ser. (Utrecht) 6: 122. 2007.
Materials examined: Australia, Queensland, Brisbane, Chapel Hill, from leaf spot of Thunbergia alata (Acanthaceae), 6 Feb. 1986, J.L. Alcorn, culture ex-type of A. thunbergiae CBS 116331 = E.G.S. 41.073 = BRIP 14963. Iran, Miandoab, from leaf of Allium cepa (Amaryllidaceae), 13 Sep. 2001, Y. Ghosta, culture ex-type of A. iranica CBS 120986 = E.G.S. 51.075. New Zealand, Auckland, Mangere, Tidal Road, from Thunbergia alata, 4 Jun. 2001, C.F. Hill, CBS 122597.

Notes: By synonymising $A$. iranica with $A$. thunbergiae, the host range of this taxon has expanded to include Allium cepa. The five sequenced genes are $100 \%$ identical between the extype strains of $A$. thunbergiae and $A$. iranica. As both species were originally described in the same publication, there is no case for nomenclatural priority. Therefore we chose to synonymise A. iranica under $A$. thunbergiae because $A$. thunbergiae is more commonly used in literature (Leahy 1992, Melo et al. 2009).

Alternaria tillandsiae E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 314. 2007.
Material examined: USA, from Tillandsia usneoides (Bromeliaceae), Dec. 1995, B. Milnes, culture ex-type of A. tillandsiae CBS 116116 = E.G.S. 43.074.

Alternaria tropica E.G. Simmons, Mycotaxon 46: 187. 1993.
Materials examined: USA, Florida, Homestead, from fruit of Passiflora edulis (Passifloraceae), May 1990, R.T. McMillan Jr., culture ex-type of A. tropica CBS 631.93 = E.G.S. 39.126; Florida, Homestead, from fruit of Passiflora edulis, May 1990, R.T. McMillan Jr., representative isolate of $A$. tropica CBS $117216=$ E.G.S. 39.125.


Fig. 27. Alternaria thunbergiae: conidia and conidiophores. A-C. CBS 116331. D-E. CBS 122597. F-H. CBS 120986. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria venezuelensis E.G. Simmons \& Rumbos, CBS Biodiversity Ser. (Utrecht) 6: 128. 2007.

Material examined: Venezuela, Maracay, from leaf spot of Phaseolus vulgaris (Fabaceae), before Oct. 1999, R. Rumbos, culture ex-type of A. venezuelensis CBS $116121=$ E.G.S. 48.065.

Alternaria zinniae M.B. Ellis, Mycol. Pap. 131: 22. 1972.
= Alternaria zinniae H. Pape, Angew. Bot. 24: 61. 1942. (nom. inval., Art. 36.1)
Materials examined: Hungary, from seed of Callistephus chinensis (Asteraceae), 12 Aug. 1942, P. Neergaard, CBS 118.44. Italy, Sardinia, Sasseri, from Zinnia elegans (Asteraceae), 18 Oct. 1958, U. Prota, CBS 117.59. Netherlands, Huizum, from leaf of Zinnia sp., 27 Jul. 1948, A. Jaarsveld, CBS 107.48. New Zealand, Auckland, Royal Oak, from leaf spot of Zinnia elegans, May 1996, C.F. Hill, representative isolate of A. zinniae CBS 117223 = E.G.S. 44.035. UK, from seed of Zinnia sp., 1979, G.S. Taylor, CBS 299.79; from seed of Zinnia sp., 1979, G.S. Taylor, CBS 300.79. Unknown, from Zinnia elegans, summer 1961, Smith, CBS 108.61.

Section Euphorbiicola Woudenb. \& Crous, sect. nov. MycoBank MB809001. Fig. 28.
Type species: Alternaria euphorbiicola E.G. Simmons \& Engelhard


Fig. 28. Alternaria section Euphorbiicola: conidia and conidiophores. A-G. Alternaria limicola. H-P. Alternaria euphorbiicola. A-D. CBS 117360. E-G. CBS 483.90. H-J. CBS 198.86. K-M. CBS 119410. $\mathrm{N}-\mathrm{P}$. CBS 133874. Scale bars $=10 \mu \mathrm{~m}$.

Section Euphorbiicola is characterised by ovoid, obclavate, medium to large conidia that are disto- and euseptate, in short to moderately long chains, with no or a simple long beak in the terminal conidia. Conidia contain multiple transverse and some longitudinal septa and are slightly constricted near some transverse septa. Short to long, broad, apical, and sometimes lateral, secondary conidiophores are formed.

Note: The new Alternaria sect. Euphorbiicola can be easily distinguished from sect. Porri based on the formation of conidia in chains in section Euphorbiicola.

Alternaria euphorbiicola E.G. Simmons \& Engelhard, Mycotaxon 25: 196. 1986.
$\equiv$ Macrosporium euphorbiae Reichert, Bot. Jahrb. Syst. 56:723.1921. Non Macrosporium euphorbiae Bartholomew 1908. (nom. illegit., Art 53.1).

Materials examined: USA, Florida, from Euphorbia pulcherrima (Euphorbiaceae), 1985, A.W. Engelhard, CBS 198.86 = E.G.S. 38.082; Hawaii, Oahu, from Euphorbia pulcherrima, Mar. 1984, M. Aragaki, representative isolate CBS 119410 = E.G.S. 41.029; Louisiana, from Euphorbia hyssopifolia (Euphorbiaceae), 1986, L. Walker, CBS 133874 = E.G.S 38.191.

Alternaria limicola E.G. Simmons \& M.E. Palm, Mycotaxon 37: 82. 1990.
Materials examined: Mexico, Colima, from leaf of Citrus aurantiifolia (Rutaceae), May 1989, M. Palm, culture ex-type of A. limicola CBS $483.90=$ E.G.S. 39.070; Jalisco, from Citrus sp., Sep. 1995, M. Palm, representative isolate CBS $117360=$ E.G.S. 43.009.

## DISCUSSION

In the present phylogenetic study aiming to delimit Alternaria species in sect. Porri, we reduced the 82 known morphospecies in this section to 63 based on our polyphasic approach. Some important plant pathogens have now been assigned to specific clades in the phylogenetic tree and correlated with their distinct morphology, which will aid plant pathologists to identify their newly collected isolates.

The 10 isolates named $A$. solani at the onset of this study cluster within five different species clades, and only three of them retain the name $A$. solani. This is not surprising, as almost all large-spored, narrow-beaked Alternaria strains hitherto isolated from Solanaceae were called A. solani, following the concept of M.B. Ellis (1971). Simmons (2000) already noted that early blight of tomato is actually caused by $A$. tomatophila rather than A. solani, and also described two additional species on tomato, A. cretica and A. subcylindrica. These tomato pathogens all cluster in one clade based on our phylogenetic analysis, which also includes the ex-type strain of $A$. linariae. The basionym of $A$. linariae, A. anagallidis var. linariae, is the oldest name in this cluster, which therefore applies to this clade mainly represented by tomato pathogens. When Neergaard (1945) described this species he found the fungus on seeds and seedlings with damping-off symptoms from Linaria marroccana (Scrophulariaceae), Antirrhinum majus (Scrophulariaceae) and on a healthy seedling of Papaver rhoeas (Papaveraceae). His pathogenicity tests (Neergaard 1945) showed that A. linariae could also attack Brassica oleracea (Brassicaceae), Solanum lycopersicum (Solanaceae), Lactuca sativa (Asteraceae), Godetia hybrida (Onagraceae), Nicotiana affinis
(Solanaceae) and Papaver paeoniflorum (Papaveraceae), indicating a very broad host range. The isolates included in this study also show that, besides its broad host range, A. linariae is also widespread, found in Europe, USA, New Zealand and Asia. Three other isolates formerly identified as $A$. solani, including a former representative isolate used by Simmons (2007), cluster with A. protenta, an Alternaria species originally described from Helianthus annuus (Asteraceae). CBS 116651 is mentioned as a representative strain of $A$. solani by Simmons (2007), but he later expressed doubt as to the identity of this isolate (Simmons pers. comm.). The host range of $A$. protenta has expanded extensively, now comprising plants from the Asteraceae, Euphorbiaceae, Gramineae and Solanaceae. A pathogenicity test performed on A. protenta isolated from sunflower seed (Wu \& Wu 2003) concluded that sunflower was the only susceptible host among the 10 host plants tested. One of the host plants tested was Solanum lycopersicum, which we include as host of $A$. protenta. However, the authors did not clearly state how the $A$. protenta isolates, which they only found on seed of one out of seven cultivars of sunflower seeds tested, were identified. The manuscript also lacks molecular data, which could affirm their identification of $A$. protenta. To our knowledge, no pathogenicity tests have thus far been performed with the species synonymised under $A$. protenta, A. hordeiseminis or A. pulcherrimae. Based on molecular and morphological data, A. protenta is closely related to $A$. solani, and these two species can only be distinguished by the 9 nt differences in their RPB2 sequences. To confirm the potato pathogen clade, called A. solani, we sequenced the RPB2 region of multiple isolates collected from Solanum tuberosum, which are present in E.G. Simmons collection, now deposited at the CBS. Almost all (22/24 strains) cluster within the now recognised $A$. solani species clade (data not shown). The ex-type strain of $A$. danida (CBS 111.44), now a synonym of $A$. solani, was originally deposited in the CBS collection by P. Neergaard as $A$. porrif. sp. solani. Pathogenicity tests performed on this strain (Neergaard 1945) showed that it could attack hosts from several plant families [e.g. Allium cepa (Amaryllidaceae), Brassica oleracea (Brassicaceae), Solanum lycopersicum (Solanaceae) and Lactuca sativa (Asteraceae)], indicating a very broad host range. Our sequences of $A$. danida differ from those deposited in GenBank by Lawrence et al. (2013), and therefore we repeated the cultivation and DNA extraction to confirm our results and the resulting synonymy with $A$. solani. Although the other large-spored, long-beaked Alternaria species described from potato, A. grandis (Simmons 2000), differs only by 1 nt in its GAPDH sequence (position 99, T instead of C, see locus alignment in TreeBASE) within the 2722 positions used in the phylogeny, we did not synonymise $A$. grandis under A. solani. The two isolates included, CBS 109158 and CBS 116695, have substantially larger conidia than the other $A$. solani isolates, and a recently published study revealed that $A$. solani (CBS 109157) and A. grandis (CBS 109158) differ on 8 out of 770 nt in their calmodulin sequence (Gannibal et al. 2014).

The oldest large-spored onion pathogens, $A$. porri and $A$. allii, form two closely related but distinct clades, which only differ based on 8 nt in their RPB2 sequences (see locus alignment in TreeBASE). The three newer species described from Allium, A. ascaloniae, A. iranica and A. vanuatuensis (Simmons 2007), are all synonymised with other species. Alternaria ascaloniae is synonymised under A. solani-nigri, a species with a broad host range, mainly found in New Zealand. To our knowledge, no pathogenicity tests have been performed with the species now placed in synonomy with A. solani-nigri, which could affirm the broad host range for this species. Alternaria iranica is synonymised under A. thunbergiae, known as the causative agent of Alternaria leaf spot on Thunbergia (Leahy 1992), reported from Australia, USA and Brazil. Alternaria vanuatuensis clusters in the Allium clade, comprising A. allii and A. porri. Based on
the sequence data generated here, it is synonymised under A. allii. According to Simmons (2007), the conidia of $A$. allii are distinguishable from those of $A$. porri and other large-spored species known on Allium, based on their multiple beaks and branches. However, the representative isolates of A. allii used by Simmons (2007) do not cluster in a single clade; CBS 116649 clusters with the two $A$. porri representative isolates. On the other hand, $A$. vanuatuensis is described as a single-beaked species, but clusters with the $A$. allii isolate deposited in the CBS collection by J.A.B. Nolla on 27 December 1927 as $A$. allii sp. nov. (CBS 107.28, recognised as the ex-type strain here). Simmons obtained this isolate from the CBS in February 2000 (E.G.S. 48.084), but was unable to induce sporulation. We observed few conidia, but these were only singlebeaked. Unfortunately we could not induce CBS 116701 to sporulate, which leaves us at odds with Simmons's notes, with only single- to double-beaked conidia in the $A$. allii clade, and double- to triple-beaked conidia in the $A$. porri clade. The number of beaks and branches from the Allium isolates therefore is not suitable to make a distinction between the two major Allium species. The species can be easily differentiated on the basis of sequence data of the RPB2 gene region generated in this study.

Based on morphology, four large-spored Alternaria species with long beaks were described as Passifloraceae pathogens. Our phylogeny only supports three of these: A. tropica, A. aragakii and the more common A. passiflorae. The fourth species, A. hawaiiensis, is synonymised under A. passiflorae based on sequence data. Simmons (2007) described $A$. hawaiiensis as a new species lacking multiple beaks, which is a characteristic of $A$. passiflorae. Our sequence data led us to conclude that this characteristic is not suitable for species delimitation, which we also concluded from the data of the onion pathogens, A. allii, A. vanuatuensis and A. porri. The clustering of two isolates within our $A$. passiflorae clade, which originate from different host families (Onagraceae and Solanaceae), renders A. passiflorae as unspecific to Passifloraceae.

An ongoing study in South Africa on sweet potato pathogens reveals multiple Alternaria species on this host associated with blight symptoms on leaves, petioles, and stems. In addition to the known pathogen of sweet potato, A. bataticola, three other pathogenic species are delineated of which two are newly described as $A$. ipomoeae and $A$. neoipomoea. A new unknown Alternaria pathogen, causing sweet potato stem blight in Ethiopia, was reported by van Bruggen in 1984. This isolate (CBS 219.79) was sent to the CBS for identification, but the author did not agree with the morphological identification made at that time as A. cucumerina, a name under which it was still stored in the CBS collection. Our data indicate that this pathogen, which also is found in stem lesions of Ipomoea batatas in South Africa, should be recognised as a new species, now named $A$. ipomoeae. Most isolates from South Africa however cluster in a clade close to $A$. ipomoeae, now named $A$. neoipomoea, which can clearly be distinguished from A. ipomoeae morphologically and by sequence data. Two more isolates from sweet potato in South Africa are identified as A. argyroxiphii, an Alternaria species originally described from Argyroxiphium sp. This finding is a new host report for A. argyroxiphii, and a first report of the fungus from South Africa.

Based on the sequence data generated in this study, A. euphorbiicola and A. limicola clearly separate from the other species in sect. Porri (Fig. 1). This separation is supported by morphological differences, and we therefore propose the new section, sect. Euphorbiicola. However, when we examined the phylogeny displaying the neighbouring sections of sect. Porri (Fig. 2), questions arose concerning sect. Gypsophilae and sect. Radicina. These two sections display almost similar branch length differences within the respective sections, comparable to what sect. Porri displays with sect. Euphorbiicola. An additional character of sect. Gypsophilae and sect. Radicina is that the species within these sections share the same
host family, respectively Caryophyllaceae and Apiaceae. We therefore choose to retain these sections at present, but additional molecular and morphological studies could eventually lead to the recognition of additional sections.

The present polyphasic approach displays the current species delimitation in Alternaria sect. Porri. We recognise 63 Alternaria species in this section with medium to large conidia and a long (filamentous) beak, which can be distinguished based on molecular data. Not all species distinctions are $100 \%$ clear based on DNA data only; nevertheless, we tried to be consistent in synonymising or not synonymising species: the number of genes with nt differences and the number of nt differences are taken into account, together with the morphology, host, country and time of isolation. All Alternaria isolates currently stored in the CBS collection, which cluster within sect. Porri based on their gene sequences, were included in our study. Some species, however, are under-sampled, which results in some uncertainty in keeping isolates as separate species or reducing them to synonymy. Although we attempted to use the available data as best as possible, with the inclusion of additional isolates some uncertain species boundaries are bound to be better resolved.

The finding of the third species on potato (A. protenta) is a good example of the importance of fungal systematics. Multiple manuscripts report on the high level of genetic variability observed among A. solani isolates (van der Waals et al. 2004, Lourenco Jr et al. 2011, Leiminger et al. 2013) and based on secondary metabolite profiling A. solani isolates cluster in two distinct groups (Andersen et al. 2008). Furthermore, two genotypes are described based on the cytochrome b gene structure of A. solani isolates (Leiminger et al. 2014), which is an important gene in fungicide resistance. However, our study indicates that previous reports could actually be dealing with three (or more) different species. Without knowing the correct identity of your pathogen, many incorrect conclusions can be drawn about diversity, evolutionary mechanisms, host range, and options for disease control.

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## Alternaria section Alternaria: species, formae speciales or pathotypes

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#### Abstract

The cosmopolitan fungal genus Alternaria consists of multiple saprophytic and pathogenic species. Based on phylogenetic and morphological studies, the genus is currently divided into 25 sections. Alternaria section Alternaria contains most of the small-spored Alternaria species with concatenated conidia, including important plant, human and postharvest pathogens. Species within section Alternaria have been mostly described based on morphology and / or host-specificity, yet molecular variation between them is minimal. To investigate whether the described morphospecies within section Alternaria are supported by molecular data, we performed whole-genome sequencing of nine Alternaria species supplemented with transcriptome sequencing of 12 Alternaria species as well as multi-gene sequencing of 168 Alternaria isolates. The assembled genomes ranged in size from 33.335.2 Mb within section Alternaria and from $32.0-39.1 \mathrm{Mb}$ for all Alternaria genomes. The number of repetitive sequences differed significantly between the different Alternaria genomes; ranging from $1.4-16.5 \%$. Within section Alternaria the repeat content is comparably low with only $1.4-2.7$ \% of repeats. Whole-genome alignments revealed 96.7-98.2 \% genome identity between section Alternaria isolates, compared to $85.1-89.3$ \% genome identity for isolates from other sections to the A. alternata reference genome. Similarly, we observed 1.4-2.8 and $0.8-1.8 \%$ single nucleotide polymorphisms (SNPs) in genomic and transcriptomic sequences, respectively, between isolates from section Alternaria, while the percentage of SNPs found in isolates from different sections compared to the A. alternata reference was considerably higher; $8.0-10.3$ and $6.1-8.5 \%$. The topology of a phylogenetic tree based on the whole-genome and transcriptome reads was congruent with multi-gene phylogenies based on commonly used gene regions. Based on the genome and transcriptome data, a set of core proteins was extracted, and primers were designed on two gene regions with a relatively low degree of conservation within section Alternaria ( 96.8 and 97.3 \% conservation). Their potential discriminatory power within section Alternaria was tested next to nine commonly used gene regions in section Alternaria, namely the SSU, LSU, ITS, GAPDH, RPB2, TEF1, Alt a 1, endoPG and OPA10-2 gene regions. We observed that the phylogenies from the two gene regions with a relatively low conservation, KOG1058 and KOG1077, could not distinguish the described morphospecies within section Alternaria more effectively than the phylogenies based on the commonly used gene regions for Alternaria. Based on genome and transcriptome comparisons and molecular phylogenies, Alternaria section Alternaria consists of only 11 phylogenetic species and one species complex. Thirty-five morphospecies, which cannot reliably be distinguished based on the multi-gene phylogeny, are synonymised under A. alternata. By providing guidelines for the naming and identification of species in Alternaria section Alternaria, we hope that this manuscript provides a clear and stable species classification in this section.


## INTRODUCTION

Alternaria section Alternaria contains most of the small-spored Alternaria species with concatenated conidia. Almost 60 morphological or host-specific species can be assigned to this section, including the type species of the genus Alternaria, A. alternata (Chapter 2). Alternaria alternata is known as the cause of leaf spot and other diseases in over 100 host species of plants (Rotem 1994), but also as post-harvest disease in various crops (Coates \& Johnson 1997) and of upper respiratory tract infections and asthma in humans (Kurup et al. 2000). Other important plant pathogens in section Alternaria include A. longipes, the causal agent of brown spot of tobacco, A. mali, the causal agent of Alternaria blotch of apple, A. gaisen, the causal agent of black spot of Japanese pear and $A$. arborescens, the causal agent of stem canker of tomato. The first descriptions of the A. alternata, A. tenuissima, A. cheiranthi and $A$. brassicicola speciesgroups, based on sporulation patterns, were made in 1995 (Simmons). More recent molecularbased studies revealed that Alternaria species cluster in several distinct species clades, now referred to as sections (Lawrence et al. 2013, Chapter 2), which do not always correlate with the species-groups that were delineated based on morphological characteristics. Currently, 25 Alternaria sections are recognised based on molecular phylogenies (Chapter 2, Chapter 4). So far, species within section Alternaria have been mostly described based on morphology and / or host-specificity; yet the molecular variation between them is minimal. The standard gene regions used for the delimitation of Alternaria species are not able to delineate species within section Alternaria (Peever et al. 2004, Andrew et al. 2009). Multiple molecular methods have been tested or proposed for separating species among the small-spored Alternaria species, including random amplified polymorphic DNA (Roberts et al. 2000), amplified fragment length polymorphism (Somma et al. 2011), selective subtractive hybridisation (Roberts et al. 2012) and sequence characterised amplified genomic regions (Stewart et al. 2013a). However, none of these methods successfully distinguished all species described within section Alternaria.

The terms forma specialis and pathotype have been used to describe isolates that are morphologically indistinguishable from A. alternata, but infect particular hosts. At least 16 different $f$. $s p$. epithets occur in the literature, of which most were raised to species level by Simmons (2007). Nishimura \& Kohmoto (1983) proposed that Alternaria strains with identical morphology but producing different host-selective toxins (HST) should be defined as distinct pathotypes of Alternaria. Currently there are seven pathotypes of $A$. alternata described (Akimitsu et al. 2014), but this term is not widely adopted, and the "old" species names are still used.

Since most morphospecies within section Alternaria cannot be distinguished based on sequences of the standard housekeeping genes (Andrew et al. 2009), whole-genome sequencing technologies can be applied to search for genes which can distinguish (most of) the described morphospecies (Lawrence et al. 2012). Since the introduction of next generation sequencing (NGS) many fungal genomes have become available for study, with the 1000 fungal genomes project (Spatafora 2011) as a public stimulant for generating this kind of data. Currently there are two publicly available Alternaria genomes at NCBI (National Center for Biotechnology Information), namely A. brassicicola, section Brassicicola (BioProject PRJNA34523) and A. arborescens, section Alternaria (BioProject PRJNA78243).

In this study, we generated whole-genome sequences of four Alternaria spp. from section Alternaria and five Alternaria spp. from five other sections, and supplemented the analyses by transcriptome sequences of nine Alternaria spp. from section Alternaria and three Alternaria spp. from three other sections of Alternaria. Species were selected based on their phylogenetic
position (Chapter 2) in such a way that they represent the whole genus Alternaria, from the sister section of section Alternaria, sect. Alternantherae (A. alternantherae), to the most distant section, sect. Crivellia (A. papaveraceae). Within section Alternaria, species were selected based on their economic importance. Based on the genome and transcriptome data, two gene regions with a relatively low conservation, the eukaryotic orthologous group (KOG) protein loci, KOG1058 ( 96.8 \% conservation) and KOG1077 (97.3 \% conservation), were identified and tested for their potential discriminatory power within section Alternaria. Together with a standard multigene phylogeny of 168 Alternaria isolates based on sequences of parts of nine gene regions, namely the internal transcribed spacer regions 1 and 2 and intervening 5.8S nrDNA (ITS), the 18 S nrDNA (SSU), the 28 S nrDNA (LSU), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), RNA polymerase second largest subunit (RPB2), translation elongation factor 1-alpha (TEF1), Alternaria major allergen gene (Alt a 1), endopolygalacturonase (endoPG) and an anonymous gene region (OPA10-2), we attempt to create a clear and stable species classification in Alternaria section Alternaria.

## MATERIALS AND METHODS

## Isolates

One-hundred-and-sixty-eight Alternaria strains, including 64 (ex-)type or representative strains, present at the Centraalbureau voor Schimmelcultures (CBS-KNAW), Utrecht, The Netherlands, were included in this study (Table 1) based on the phylogenetic position derived from their ITS sequence. With 'representative isolate' we refer to the strains used to describe the species based on morphology in The Alternaria Identification Manual (Simmons 2007). Freeze-dried strains were revived in 2 ml malt / peptone ( $50 \% / 50 \%$ ) and subsequently transferred to oatmeal agar (OA) (Crous et al. 2009c). Strains stored in liquid nitrogen were transferred to OA directly from the $-185^{\circ} \mathrm{C}$ storage.

## DNA and RNA isolation for NGS

The genomes of four Alternaria spp. from section Alternaria and five Alternaria spp. from five other sections (Table 2) as well as the transcriptome profiles of nine Alternaria spp. from section Alternaria and three Alternaria spp. representing three other sections of Alternaria were sequenced (Table 3). Species were selected based on their economic importance and their phylogenetic position, with the intention to be representative of the entire genus Alternaria with a focus on section Alternaria. Isolates were grown in malt peptone (MP) (Crous et al. 2009c) supplemented with $1 \times$ BME vitamin solution (Sigma-Aldrich ${ }^{\circledR}$ Chemie B.V., Zwijndrecht, The Netherlands) in a shaking incubator, at $25^{\circ} \mathrm{C}$, in the dark, for 3 d . When growth was observed, cultures were mixed in a blender and transferred to fresh MP with vitamin solution, and returned to the shaking incubator for another 2-3 d. When sufficient growth was observed, the mycelium was harvested with a Whatman No. 4 filter disk and a Buchner funnel, attached to a vacuum flask.

For isolating DNA, QIAGEN Genomic 100/G tips (QIAGEN Benelux B.V., Venlo, The Netherlands) were used and processed following the lysis protocol for tissue in the QIAGEN Blood \& Cell Culture DNA kit. The following alternative steps, as suggested by the protocol, were followed. The mycelium, of which a maximum of 4 g (wet weight) was used, was grinded to a fine powder with liquid nitrogen in a pre-cooled mortar and pestle. Proteinase K stock
solution was added to the solution, after which it was incubated for 2 h at $50^{\circ} \mathrm{C}$ in a shaking incubator running at 700 rpm . Prewarmed QF buffer $\left(50^{\circ} \mathrm{C}\right)$ was used to elute the genomic DNA, and after precipitation the DNA was centrifuged at $4^{\circ} \mathrm{C}$ for 20 min at $8500 \times \mathrm{g}$.

For isolating RNA, the QIAGEN RNeasy Midi kit was used following the protocol for isolation of total RNA from animal tissues including the optional on-column DNase digestion. For the disruption of the tissue and homogenisation of the lysate, the mortar and pestle with needle and syringe homogenisation method, as described in the protocol, was followed. All centrifuge steps are performed at room temperature at $4000 \times \mathrm{g}$. When necessary, a final standard LiCl purification was performed.

## NGS

DNA sequence and RNA sequence library preparation ( 500 bp insert) for Illumina ${ }^{\circledR}$ sequencing and the sequencing itself(100-bp paired end reads) were performed at the Applied Biosystematics group of Plant Research International (PRI, Wageningen, The Netherlands).

DNA sequence library preparation for Ion Torrent ${ }^{\mathrm{TM}}$ sequencing was performed at the CBSKNAW. The Ion Torrent ${ }^{\mathrm{TM}}$ library preparation was carried out using the Ion Xpress ${ }^{\mathrm{TM}}$ Fragment Library Kit (Thermo Fisher Scientific, Bleiswijk, The Netherlands), with 180 ng of DNA. Adapter ligation, size selection and nick repair were performed as described in the Ion Torrent ${ }^{\mathrm{TM}}$ protocol using the Ion Xpress ${ }^{\mathrm{TM}}$ Plus Fragment Library Kit (Thermo Fisher Scientific), with a shearing time of 13 min . The 2100 Bioanalyzer system (Agilent Technologies Netherlands BV, Amstelveen, The Netherlands) and the associated High Sensitivity DNA Analysis kit (Agilent Technologies) were used to determine the quality and concentration of the libraries. The amount of library required for template preparation was calculated using the Template Dilution Factor calculation described in the protocol (DNA concentration diluted to 42 pM ). Emulsion PCR and enrichment steps were carried out using the Ion PGM ${ }^{\mathrm{TM}}$ Template OT2 200 Kit (Thermo Fisher Scientific) and associated protocol. The enrichment percentage was determent via the Ion Sphere ${ }^{\mathrm{TM}}$ Quality Control Kit (Thermo Fisher Scientific) and was performed between the emulsion PCR and the enrichment step. Sequencing was performed using the Ion PGM ${ }^{\mathrm{TM}}$ Sequencing 200 Kit v2 (Thermo Fisher Scientific) with an Ion $318^{\mathrm{TM}}$ Chip Kit v2 (Thermo Fisher Scientific).

## Genome assembly and mapping

De novo genome assembly of the Illumina ${ }^{\circledR}$ paired-end reads were quality-filtered and assembled using the A5 pipeline v. 13.01.2014 (Tritt et al. 2012) and de novo genome assembly of Ion Torrent ${ }^{\mathrm{TM}}$ reads was performed using Newbler v. 2.9 ( 454 Life Sciences, Roche Applied Science, Branford, CT, USA). Repeats in the assembled genomes were identified using de novo repeat detection with RepeatModeler (Smit \& Hubley 2008) followed by genome-wide repeat annotation using RepeatMasker (Smit et al. 1996), combining the de novo repeats with previously described repeat families from RepBase Update (release 31-04-2014) (Jurka et al. 2005).

Whole-genome alignments were performed using NUCmer, part of the MUMmer v. 3.1 package (Kurtz et al. 2004), using the 'mum' option to find matches unique in query and reference. Subsequently, the average identity of the aligned sequences was calculated using dnadiff, part of MUMmer v. 3.1.

Genomic variants were inferred using GATK v. 3.3 (DePristo et al. 2011). Briefly, genomic or transcriptomic reads were mapped against a reference genome (A. alternata CBS 916.96)
Table 1. Isolates used in this study and their GenBank accession numbers. Species name and strain number ${ }^{1,2}$

## Locality, host / substrate

GenBank accession numbers ${ }^{3}$

| KOG1058 | KOG1077 |
| :--- | :---: |
| KP125226 | np |
|  |  |
| KP125227 | KP125275 |


| KC584506 | KC584251 | KC584179 | KC584096 | KC584633 | KC584374 | KP123846 | np | np | KP125227 | KP125275 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| KP124919 | KP124449 | KP124298 | KP124155 | KP125073 | KP124766 | KP123847 | AY295020 | JQ800620 |  |  |
| KP124920 | KP124450 | KP124299 | KP124156 | KP125074 | KP124767 | KP123848 | KP123995 | KP124603 |  |  |
| KP124921 | KP124451 | KP124300 | KP124157 | KP125075 | KP124768 | KP123849 | KP123996 | KP124604 |  |  |
| KP124922 | KP124452 | KP124301 | KP124158 | KP125076 | KP124769 | KP123851 | KP123998 | KP124606 |  |  |
| KP124923 | KP124453 | KP124302 | KP124159 | KP125077 | KP124770 | KP123852 | KP123999 | KP124607 | KP125228 | KP125276 |
| KP124924 | KP124454 | Y17071 | JQ646308 | KP125078 | KP124771 | KP123853 | KP124000 | KP124608 |  |  |

KP124610
KP124611
KP124612
KP124613
KP124614
$n p$
np
Table 1. (Continued).

| $\text { number }{ }^{1,2}$ | Locality, host / substrate |  |  |  |  | GenBank | accession | numbers ${ }^{3}$ |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | SSU | LSU | ITS | GAPDH | TEF1 | RPB2 | Alt a 1 | endoPG | OPA10-2 | KOG1058 | KOG1077 |
| CBS 612.72; DSM 62012 (A. cinerariae) | Germany, Senecio cineraria | KP124930 | KP124460 | KP124308 | KP124165 | KP125084 | KP124777 | KP123861 | KP124008 | KP124615 |  |  |
| CBS 795.72; ATCC 24127; IHEM 3789 | USA, Plantago aristida | KP124931 | KP124461 | KP124309 | KP124166 | KP125085 | KP124778 | KP123862 | KP124009 | KP124616 |  |  |
| CBS 198.74 (A. chlamydospora) | Kuwait, soil | KP124932 | KP124462 | KP124310 | KP124167 | KP125086 | np | KP123863 | KP124010 | KP124617 |  |  |
| CBS 267.77 (A. citri) | USA, Citrus paradisi | KP124933 | KP124463 | KP124311 | KP124168 | KP125087 | KP124779 | KP123864 | KP124011 | KP124618 |  |  |
| CBS 603.78; E.G.S. 30.134; QM 9553 | USA, air | KP124934 | KP124464 | KP124312 | KP124169 | KP125088 | KP124780 | KP123865 | KP124012 | KP124619 |  |  |
| CBS 175.80 (A. septorioides) | Italy, unknown | KP124935 | KP124465 | KP124313 | JQ646324 | KP125089 | KP124781 | KP123866 | KP124013 | KP124620 |  |  |
| CBS 192.81 (A. citri) | Egypt, Citrus sinensis | KP124936 | KP124466 | KP124314 | KP124170 | KP125090 | KP124782 | KP123867 | KP124014 | KP124621 |  |  |
| CBS 620.83; ATCC 15052 <br> (A. tenuissima) | USA, Nicotiana tabacum | KP124937 | KP124467 | KP124315 | KP124171 | KP125091 | KP124783 | KP123868 | KP124015 | KP124622 |  |  |
| CBS 194.86; E.G.S. 04.090; QM 1347 (A. pulvinifungicola ${ }^{\text {T }}$ ) | USA, Quercus sp. | KP124938 | KP124468 | KP124316 | KP124172 | KP125092 | KP124784 | KP123869 | KP124016 | KP124623 | KP125230 | KP125278 |
| CBS 195.86; E.G.S. <br> 36.172; DAOM 185214 (A. angustiovoidea ${ }^{\text {T }}$ ) | Canada, Euphorbia esula | KP124939 | KP124469 | KP124317 | KP124173 | KP125093 | KP124785 | JQ646398 | KP124017 | KP124624 | KP125231 | KP125279 |
| CBS 447.86 (A. malvae) | Marocco, Malva sp. | KP124940 | KP124470 | KP124318 | JQ646314 | KP125094 | KP124786 | JQ646397 | KP124018 | KP124625 |  |  |
| CBS 479.90; E.G.S. 29.028 <br> (A. pellucida ${ }^{\mathrm{T}}$ ) | Japan, Citrus unshiu | KP124941 | KP124471 | KP124319 | KP124174 | KP125095 | KP124787 | KP123870 | KP124019 | KP124626 | KP125232 | KP125280 |
| CBS 595.93 (A. rhadina ${ }^{\text {T }}$ ) | Japan, Pyrus pyrifolia | KP124942 | KP124472 | KP124320 | KP124175 | KP125096 | KP124788 | JQ646399 | KP124020 | KP124627 |  |  |
| CBS 877.95 (A. tenuissima) | India, human, sinusitis | KP124943 | KP124473 | KP124321 | KP124176 | KP125097 | KP124789 | KP123871 | KP124021 | np |  |  |
| CBS 880.95; IMI 292915 (A. tenuissima) | Belgium, Fragaria vesca | KP124944 | KP124474 | KP124322 | KP124177 | KP125098 | KP124790 | np | KP124022 | KP124628 |  |  |
| CBS 965.95; IMI 289679 (A. tenuissima) | India, Triticum sp. | KP124945 | KP124475 | KP124323 | KP124178 | KP125099 | KP124791 | KP123872 | KP124023 | KP124629 |  |  |
| CBS 966.95; IMI 79630 (A. tenuissima) | India, Solanum lycopersicum | KP124946 | KP124476 | KP124324 | KP124179 | KP125100 | KP124792 | KP123873 | KP124024 | KP124630 |  |  |
| CBS 806.96 | Papua New Guinea, Cyperaceae | KP124947 | KP124477 | KP124325 | KP124180 | KP125101 | KP124793 | KP123874 | KP124025 | KP124631 |  |  |

Table 1. (Continued).
Species name and strain
number ${ }^{1,2}$
Locality, host / substrate
CBS 916.96; E.G.S. 34.016;
CBS 110977; CBS 115616; Arachis hypogaea
IMI 254138
CBS 918.96; E.G.S. 34.015; UK, Dianthus chinensis
IMI 255532 (A. tenuissima
CBS 911.97 ; IMI 056271 (A. India, Artemisia brevifolia
tenuissima)
CBS 639.97; IMI $366417 \quad$ Greece, Helianthus annuus
CBS 102595; E.G.S. 45.100 USA, Citrus jambhiri
,
CBS 102596; E.G.S (A. citrimacularis ${ }^{T}$ )
CBS 102598; E.G (A. citriarbusti ${ }^{\mathrm{T}}$ )
CBS $1025\left({ }^{\text {a }}{ }^{\text {T }}\right.$
E.G.S.
CBS 102600, 38963 39.181; ATCC
toxicogenica ${ }^{\text {T }}$
toxicogenica ${ }^{\text {) }}$
(AS 102602; E.
(A. perangusta
(A. interrupta ${ }^{\mathrm{T}}$ )
(A.
CBS 109455
CBS 109803 CBS 110027 Germany, human eye CBS 110977; E.G.S. 34.016; India, Arachis hypogaea CBS 916.96; CBS 115616 ${ }^{\text {² }}$
CBS 112249
Unknown, unknown Unknown, unknown Unknown, unknown CBS 112252 (A. tenuissima)
Table 1. (Continued).

| number ${ }^{1,2}$ | Locality, host / substrate | GenBank accession numbers ${ }^{3}$ |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | SSU | LSU | ITS | GAPDH | TEF1 | RPB2 | Alt a 1 | endoPG | OPA10-2 | KOG1058 | KOG1077 |
| CBS 113013; CPC 4268 (A. tenuissima) | South Africa, Malus domestica | KP124963 | KP124493 | KP124341 | KP124195 | KP125117 | KP124809 | KP123889 | KP124042 | KP124651 |  |  |
| CBS 113014; CPC 4260 (A. tenuissima) | South Africa, Malus domestica | KP124964 | KP124494 | KP124342 | KP124196 | KP125118 | KP124810 | KP123890 | KP124043 | KP124652 |  |  |
| CBS 113015; CPC 4266 ( $A$. tenuissima) | South Africa, Malus domestica | KP124965 | KP124495 | KP124343 | KP124197 | KP125119 | KP124811 | KP123891 | KP124044 | KP124653 |  |  |
| CBS 113024; CPC 4334 | South Africa, Minneola tangelo | KP124966 | KP124496 | KP124344 | KP124198 | KP125120 | KP124812 | KP123892 | KP124045 | KP124654 |  |  |
| CBS 113025; CPC 4342 | South Africa, Citrus clementina | KP124967 | KP124497 | KP124345 | KP124199 | KP125121 | KP124813 | KP123893 | KP124046 | KP124655 |  |  |
| CBS 113054; CPC 4263 (A. tenuissima) | South Africa, Malus domestica | KP124968 | KP124498 | KP124346 | KP124200 | KP125122 | KP124814 | KP123894 | KP124047 | KP124656 |  |  |
| CBS 115069; CPC 4254 (A. tenuissima) | South Africa, Malus domestica | KP124969 | KP124499 | KP124347 | KP124201 | KP125123 | KP124815 | KP123895 | KP124048 | KP124657 |  |  |
| CBS 115152; HKUCC 9099 | China, Psychotria serpens | KP124970 | KP124500 | KP124348 | KP124202 | KP125124 | KP124816 | KP123896 | KP124049 | KP124658 |  |  |
| CBS 115188; CPC 4348 | South Africa, Citrus clementina | KP124971 | KP124501 | KP124349 | KP124203 | KP125125 | KP124817 | KP123897 | KP124050 | KP124659 |  |  |
| CBS 115190; CPC 4340 | South Africa, Citrus sinensis | KP124972 | KP124502 | KP124350 | KP124204 | KP125126 | KP124818 | KP123898 | KP124051 | KP124660 |  |  |
| CBS 115199; CPC 4327 | South Africa, Minneola tangelo | KP124973 | KP124503 | KP124351 | KP124205 | KP125127 | KP124819 | KP123899 | KP124052 | KP124661 |  |  |
| CBS 115200; CPC 4325 | South Africa, Minneola tangelo | KP124974 | KP124504 | KP124352 | KP124206 | KP125128 | KP124820 | KP123900 | KP124053 | KP124662 |  |  |
| CBS 115616; EGS 34.016; CBS 916.96; CBS $110977^{\text {T }}$ | India, Arachis hypogaea | KC584507 | DQ678082 | AF347031 | AY278808 | KC584634 | KC584375 | AY563301 | JQ811978 | KP124663 |  |  |
| CBS 116749 | Netherlands, unknown | KP124975 | KP124505 | KP124353 | KP124207 | KP125129 | KP124821 | KP123901 | KP124054 | KP124664 |  |  |
| CBS 117130 | Italy, Arbutus unedo | KP124976 | KP124506 | KP124354 | KP124208 | KP125130 | KP124822 | KP123902 | KP124055 | KP124665 |  |  |
| CBS 117143 | Italy, Capsicum annuum | KP124977 | KP124507 | KP124355 | KP124209 | KP125131 | KP124823 | KP123903 | KP124056 | KP124666 |  |  |
| CBS 118811; E.G.S. 35.158 <br> (A. brassicinae ${ }^{\mathrm{T}}$ ) | USA, Brassica oleracea | KP124978 | KP124508 | KP124356 | KP124210 | KP125132 | KP124824 | KP123904 | KP124057 | KP124667 | KP125242 | KP125290 |
| CBS 118812; E.G.S. 37.050 (A. daucifoliii) | USA, Daucus carota | KC584525 | KC584269 | KC584193 | KC584112 | KC584652 | KC584393 | KP123905 | KP124058 | KP124668 | KP125243 | KP125291 |

Table 1. (Continued).
Species name and strain
number ${ }^{1,2}$

GenBank accession numbers ${ }^{3}$

- OPA10-2 KOG1058 KOG1077 KP125244 KP125292
LPI25293

KP125249 KP125297
np
KP124672
KP124673
P124674
KP124675
KP124676
677
KP124068 KP124677
KP124069 KP124678

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KP124682

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KP124509
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KP124062
KP124063
KP124064

KP 124066






KP124839 KP123917

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KP124359 KP124213
KP124214
JQ646328
JQ646326
UP124215
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KP124217
KP124218
KP124219
AY278812

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KP125147

RP1362
KP124363
KP124364
KJ862254
KP124365
KP124366
KP124367
AF278836
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KP124519
KP124520
KP124991 KP124521
KP124522

SSU
CBS 118814; E.G.S. 44.048 USA, Solanum lycopersicum KP124979
KP124980
KP124981
KP124982
KP124983
KP124984
KP124985
KP124986
KP124987
KP124988
KP124988
KP124989
KP124990
KP124992
KP124993
Greece, Punica granatum USA, Allium sp.
Israel, Minneola tangelo
South Africa, Minneola tangelo
China, Platycodon
grandiflorus
USA, Cuscuta gronovii
China, Broussonetia papyrifera
 officinalis
KP124994
KP124995
KP124996
Table 1. (Continued).

| $\text { number }{ }^{1,2}$ | Locality, host / substrate | GenBank accession numbers ${ }^{3}$ |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | SSU | LSU | ITS | GAPDH | TEF1 | RPB2 | Alt a 1 | endoPG | OPA10-2 | KOG1058 | KOG1077 |
| CBS 124277 (A. tenuissima) | Denmark, Prunus sp. | KP124997 | KP124527 | KP124373 | KP124225 | KP125151 | KP124843 | KP123921 | KP124077 | KP124686 |  |  |
| CBS 124278 (A. tenuissima) | Denmark, Prunus sp. | KP124998 | KP124528 | KP124374 | KP124226 | KP125152 | KP124844 | KP123922 | KP124078 | KP124687 |  |  |
| CBS 125606 | India, human | KP124999 | KP124529 | KP124375 | KP124227 | KP125153 | KP124845 | KP123923 | KP124079 | KP124688 |  |  |
| CBS 126071 (A. tenuissima) | Namibia, soil | KP125000 | KP124530 | KP124376 | KP124228 | KP125154 | KP124846 | KP123924 | KP124080 | KP124689 |  |  |
| CBS 126072 (A. tenuissima) | Namibia, soil | KP125001 | KP124531 | KP124377 | KP124229 | KP125155 | KP124847 | KP123925 | KP124081 | KP124690 |  |  |
| CBS 126908 | USA, soil | KP125002 | KP124532 | KP124378 | KP124230 | KP125156 | KP124848 | KP123926 | KP124082 | KP124691 |  |  |
| CBS 126910 (A. tenuis) | USA, soil | KP125003 | KP124533 | KP124379 | KP124231 | KP125157 | KP124849 | KP123927 | KP124083 | KP124692 |  |  |
| CBS 127334 | USA, soil | KP125004 | KP124534 | KP124380 | KP124232 | KP125158 | KP124850 | KP123928 | KP124084 | KP124693 |  |  |
| CBS 127671; E.G.S. 52.121 <br> (A. seleniiphila ${ }^{\mathrm{T}}$ ) | USA, Stanleya pinnata | KP125005 | KP124535 | KP124381 | KP124233 | KP125159 | KP124851 | KP123929 | KP124085 | KP124694 |  |  |
| CBS 127672; E.G.S. 52.122 <br> (A. astragali ${ }^{\mathrm{T}}$ ) | USA, Astragalus bisulcatus | KP125006 | KP124536 | KP124382 | KP124234 | KP125160 | KP124852 | KP123930 | KP124086 | KP124695 |  |  |
| CBS 130254 | India, human sputum | KP125007 | KP124537 | KP124383 | KP124235 | KP125161 | KP124853 | KP123931 | KP124087 | KP124696 |  |  |
| CBS 130255 | India, human sputum | KP125008 | KP124538 | KP124384 | KP124236 | KP125162 | KP124854 | KP123932 | KP124088 | KP124697 |  |  |
| CBS 130258 | India, human sputum | KP125009 | KP124539 | KP124385 | KP124237 | KP125163 | KP124855 | KP123933 | KP124089 | KP124698 |  |  |
| CBS 130259 | India, human sputum | KP125010 | KP124540 | KP124386 | KP124238 | KP125164 | KP124856 | KP123934 | KP124090 | KP124699 |  |  |
| CBS 130260 | India, human sputum | KP125011 | KP124541 | KP124387 | KP124239 | KP125165 | KP124857 | KP123935 | KP124091 | KP124700 |  |  |
| CBS 130261 | India, human sputum | KP125012 | KP124542 | KP124388 | KP124240 | KP125166 | KP124858 | KP123936 | KP124092 | KP124701 |  |  |
| CBS 130262 | India, human sputum | KP125013 | KP124543 | KP124389 | KP124241 | KP125167 | KP124859 | KP123937 | KP124093 | KP124702 |  |  |
| CBS 130263 | India, human sputum | KP125014 | KP124544 | KP124390 | KP124242 | KP125168 | KP124860 | KP123938 | KP124094 | KP124703 |  |  |
| CBS 130265 | India, human sputum | KP125015 | KP124545 | KP124391 | KP124243 | KP125169 | KP124861 | KP123939 | KP124095 | KP124704 |  |  |
| Alternaria arborescens SC |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 101.13; E.G.S. 07.022; <br> QM1765 (A. geophila ${ }^{\text {T }}$ ) | Switzerland, peat soil | KP125016 | KP124546 | KP124392 | KP124244 | KP125170 | KP124862 | KP123940 | KP124096 | KP124705 | KP125254 | KP125302 |
| CBS 105.24; IHEM 3123 ( $A$. alternata) | Unknown, Solanum tuberosum | KP125017 | KP124547 | KP124393 | KP124245 | KP125171 | KP124863 | KP123941 | KP124097 | KP124706 |  |  |
| CBS 108.41; E.G.S. 44.087; <br> ATCC 11892 (A. alternata) | Unknown, wood | KP125018 | KP124548 | KP124394 | KP124246 | KP125172 | KP124864 | KP123942 | KP124098 | KP124707 |  |  |
| CBS 113.41; IHEM 3318 (A. alternata) | Unknown, Schizanthus sp. | KP125019 | KP124549 | KP124395 | KP124247 | KP125173 | KP124865 | KP123943 | KP124099 | KP124708 |  |  |

Table 1．（Continued）．
Species name and strain
number ${ }^{1,2}$
Locality，host／substrate
GenBank accession numbers ${ }^{3}$
endoPG OPA10－2 KOG1058 KOG1077 KP124100 KP124709
TP124710
KP124102 KP12471
KP124712
KP124713
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KP124715
KP124106 KP124716
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KP124109 KP124719

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KP125177 KP124869 KP123946

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KP124249
KP124250
KP124251
KP124252
KP124253
KP124405 KP124257
KP124406 KP124258
KP124407 KP124259
JQ646321


KP125188 | $\infty$ |
| :---: |
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| $\frac{\pi}{2}$ |
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Table 1. (Continued).

| number ${ }^{1,2}$ | Locality, host / substrate | GenBank accession numbers ${ }^{3}$ |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | SSU | LSU | ITS | GAPDH | TEF1 | RPB2 | Alt a 1 | endoPG | OPA10-2 | KOG1058 | KOG1077 |
| $\text { CBS } 124281 \text { (A. }$ arborescens) | Denmark, Triticum sp. | KP125037 | KP124567 | KP124414 | KP124265 | KP125192 | KP124883 | KP123961 | KP124118 | KP124728 |  |  |
| CBS 124282 ( $A$. arborescens) | Denmark, Hordeum vulgare | KP125038 | KP124568 | KP124415 | KP124266 | KP125193 | KP124884 | KP123962 | KP124119 | KP124729 |  |  |
| CBS 124283 (A. tenuissima) | Russia, Oryza sp. | KP125039 | KP124569 | KP124416 | KP124267 | KP125194 | KP124885 | KP123963 | KP124120 | KP124730 |  |  |
| CBS 127263 (A. alternata) | Mexico, human nasal infection | KP125040 | KP124570 | KP124417 | KP124268 | KP125195 | KP124886 | KP123964 | KP124121 | KP124731 |  |  |
| CPC 25266 | Austria, Pyrus sp. | KP125041 | KP124571 | KP124418 | KP124269 | KP125196 | KP124887 | KP123965 | KP124122 | KP124732 |  |  |
| Alternaria betae-kenyensis |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 118810; E.G.S. 49.159; IMI $385709^{\text {T }}$ | Kenya, Beta vulgaris var. cicla | KP125042 | KP124572 | KP124419 | KP124270 | KP125197 | KP124888 | KP123966 | KP124123 | KP124733 | KP125258 | KP125306 |
| Alternaria burnsii |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 108.27 | Unknown, Gomphrena globosa | KC584601 | KC584343 | KC584236 | KC584162 | KC584727 | KC584468 | KP123850 | KP123997 | KP124605 |  |  |
| CBS 107.38; E.G.S. $06.185^{\text {T }}$ | India, Cuminum cyminum | KP125043 | KP124573 | KP124420 | JQ646305 | KP125198 | KP124889 | KP123967 | KP124124 | KP124734 | KP125259 | np |
| CBS 110.50; MUCL 10012 <br> (A. gossypina) | Mozambique, Gossypium sp. | KP125044 | KP124574 | KP124421 | KP124271 | KP125199 | KP124890 | KP123968 | KP124125 | KP124735 |  |  |
| CBS 879.95; IMI 300779 (A. tenuissima) | UK, Sorghum sp. | KP125045 | KP124575 | KP124422 | KP124272 | KP125200 | KP124891 | KP123969 | KP124126 | KP124736 |  |  |
| CBS 118816; E.G.S. <br> 43.145; IMI 368045 ( $A$. rhizophorae ${ }^{\mathrm{T}}$ ) | India, Rhizophora mucronata | KP125046 | KP124576 | KP124423 | KP124273 | KP125201 | KP124892 | KP123970 | KP124127 | KP124737 | KP125260 | KP125307 |
| CBS 118817; E.G.S. 39.014; IMI 318433 (A. tinosporae ${ }^{\mathrm{T}}$ ) | India, Tinospora cordifolia | KP125047 | KP124577 | KP124424 | KP124274 | KP125202 | KP124893 | KP123971 | KP124128 | KP124738 | KP125261 | KP125308 |
| CBS 130264 | India, human sputum | KP125048 | KP124578 | KP124425 | KP124275 | KP125203 | KP124894 | KP123972 | KP124129 | KP124739 |  |  |
| Alternaria eichhorniae |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 489.92; ATCC 22255; ATCC 46777; IMI $121518^{\text {T }}$ | India, Eichhornia crassipes | KP125049 | KP124579 | KC146356 | KP124276 | KP125204 | KP124895 | KP123973 | KP124130 | KP124740 | KP125262 | KP125309 |
| CBS 119778; E.G.S. 45.026; IMI $37968^{\mathrm{R}}$ | Indonesia, Eichhornia crassipes | KP125050 | KP124580 | KP124426 | KP124277 | KP125205 | KP124896 | np | KP124131 | KP124741 | KP125263 | KP125310 |
| Alternaria gaisen |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 632.93; E.G.S. $90.512^{\text {R }}$ | Japan, Pyrus pyrifolia | KC584531 | KC584275 | KC584197 | KC584116 | KC584658 | KC584399 | KP123974 | AY295033 | KP124742 | KP125264 | KP125311 |

Table 1. (Continued).

| number ${ }^{1,2}$ | Locality, host / substrate | GenBank accession numbers ${ }^{3}$ |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | SSU | LSU | ITS | GAPDH | TEF1 | RPB2 | Alt a 1 | endoPG | OPA10-2 | KOG1058 | KOG1077 |
| CBS 118488; E.G.S. $90.391^{\text {R }}$ | Japan, Pyrus pyrifolia | KP125051 | KP124581 | KP124427 | KP124278 | KP125206 | KP124897 | KP123975 | KP124132 | KP124743 | KP125265 | KP125312 |
| CPC 25268 | Portugal, unknown | KP125052 | KP124582 | KP124428 | KP124279 | KP125207 | KP124898 | KP123976 | KP124133 | KP124744 |  |  |
| Alternaria gossypina |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 100.23 (A. grossulariae) | Unknown, Malus domestica | KP125053 | KP124583 | KP124429 | KP124280 | KP125208 | KP124899 | KP123977 | KP124134 | KP124745 |  |  |
| CBS 104.32 ${ }^{\text {T }}$ | Zimbabwe, Gossypium sp. | KP125054 | KP124584 | KP124430 | JQ646312 | KP125209 | KP124900 | JQ646395 | KP124135 | KP124746 |  |  |
| CBS 107.36 (A. grisea ${ }^{\text {T }}$ ) | Indonesia, soil | KP125055 | KP124585 | KP124431 | JQ646310 | KP125210 | KP124901 | JQ646393 | KP124136 | KP124747 |  |  |
| CBS 102597; E.G.S. 45.114 <br> (A. tangelonis ${ }^{\text {T }}$ ) | USA, Minneola tangelo | KP125056 | KP124586 | KP124432 | KP124281 | KP125211 | KP124902 | KP123978 | KP124137 | KP124748 | KP125266 | KP125313 |
| CBS 102601; E.G.S. 45.017 <br> (A. colombiana ${ }^{\text {T }}$ ) | Colombia, Minneola tangelo | KP125057 | KP124587 | KP124433 | KP124282 | KP125212 | KP124903 | KP123979 | KP124138 | KP124749 | KP125267 | KP125314 |
| Alternaria iridiaustralis |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 118404; E.G.S. 49.078; MAFF $354 \mathrm{~A}^{\mathrm{R}}$ | New Zealand, Iris sp. | KP125058 | KP124588 | KP124434 | KP124283 | KP125213 | KP124904 | KP123980 | KP124139 | KP124750 | KP125268 | np |
| CBS 118486; E.G.S. $43.014^{\text {T }}$ | Australia, Iris sp. | KP125059 | KP124589 | KP124435 | KP124284 | KP125214 | KP124905 | KP123981 | KP124140 | KP124751 |  |  |
| CBS 118487; E.G.S. $44.147^{\text {R }}$ | Australia, Iris sp. | KP125060 | KP124590 | KP124436 | KP124285 | KP125215 | KP124906 | KP123982 | KP124141 | KP124752 |  |  |
| Alternaria jacinthicola |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 878.95; IMI 77934b ( $A$. tenuissima) | Mauritius, Arachis hypogaea | KP125061 | KP124591 | KP124437 | KP124286 | KP125216 | KP124907 | KP123983 | KP124142 | KP124753 | KP125269 | np |
| CBS 133751; MUCL 53159 ${ }^{\text {T }}$ | Mali, Eichhornia crassipes | KP125062 | KP124592 | KP124438 | KP124287 | KP125217 | KP124908 | KP123984 | KP124143 | KP124754 | KP125270 | np |
| CPC 25267 | Unknown, Cucumis melo var. inodorus | KP125063 | KP124593 | KP124439 | KP124288 | KP125218 | KP124909 | KP123985 | KP124144 | KP124755 | KP125271 | np |
| Alternaria longipes |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 113.35 | Unknown, Nicotiana tabacum | KP125064 | KP124594 | KP124440 | KP124289 | KP125219 | KP124910 | KP123986 | KP124145 | KP124756 |  |  |
| CBS 539.94; QM 8438 | USA, Nicotiana tabacum | KP125065 | KP124595 | KP124441 | KP124290 | KP125220 | KP124911 | KP123987 | KP124146 | KP124757 |  |  |
| CBS 540.94; E.G.S. 30.033; QM $9589^{R}$ | USA, Nicotiana tabacum | KC584541 | KC584285 | AY278835 | AY278811 | KC584667 | KC584409 | AY563304 | KP124147 | KP124758 | KP125272 | KP125315 |
| CBS 917.96 | USA, Nicotiana tabacum | KP125066 | KP124596 | KP124442 | KP124291 | KP125226 | KP124912 | KP123988 | KP124148 | KP124759 |  |  |
| CBS 121332; E.G.S. $30.048^{\text {R }}$ | USA, Nicotiana tabacum | KP125067 | KP124597 | KP124443 | KP124292 | KP125227 | KP124913 | KP123989 | KP124149 | KP124760 |  |  |
| CBS 121333; E.G.S. $30.051^{\text {R }}$ | USA, Nicotiana tabacum | KP125068 | KP124598 | KP124444 | KP124293 | KP125223 | KP124914 | KP123990 | KP124150 | KP124761 |  |  |
| Alternaria tomato |  |  |  |  |  |  |  |  |  |  |  |  |
| CBS 103.30 | Unknown, Solanum lycopersicum | KP125069 | KP124599 | KP124445 | KP124294 | KP125224 | KP124915 | KP123991 | KP124151 | KP124762 | KP125273 | KP125316 |

Table 1. (Continued).

ATCC: American Type Culture Collection, Manassas, VA, USA; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Personal collection of P.W. Crous, Utrecht, The Netherlands; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; DSM: German Collection of Microorganisms and Cell Cultures, Leibniz Institute, Braunschweig, Germany; E.G.S.: Personal collection of Dr. E.G. Simmons; HKUCC: The University of Hong Kong Culture Collection, Hong Kong, China; HSAUP: Department of Plant Pathology, Shandong Agricultural University, China; IFO: Institute for Fermentation Culture Collection, Osaka, Japan; IHEM: Biomedical Fungi and Yeast Collection of the Belgian Co-ordinated Collections of Micro-organisms (BCCM), Brussels, Belgium; IMI: Culture collection of CABI Europe UK Centre, Egham UK; LCP: Laboratory of Cryptogamy, National Museum of Natural History, Paris, France; MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisherie, Tsukuba, Japan; MUCL: (Agro)Industrial Fungi and Yeast Collection of the Belgian Co-ordinated Collections of Micro-organisms (BCCM), Louvain-la-Neuve, Belgium; QM: Quarter Master Culture Collection, Amherst, MA, USA; VKM: All-Russian Collection of Microorganisms, Moscow, Russia.
${ }^{2}$ T: ex-type isolate; R: representative isolate; Species names between parentheses refer to the former species name. ${ }^{3}$ Bold accession numbers are generated in other studies; np: no product.
Table 2. Assembly statistics of the Alternaria genomes.

| Species | Strain number | Section | Sequencing method | Size (Mb) | Coverage (approx.) | \% Repeats | \% Identity | \% SNPs ${ }^{2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A. alternata | CBS 916.96 ${ }^{3}$ | Alternaria | Illumina | 33.3 | $40 \times$ | 1.4 | na ${ }^{3}$ | na ${ }^{3}$ |
| A. arborescens ${ }^{\text {l }}$ | E.G.S. $39.128=$ CBS 102605 | Alternaria | - | 33.9 | - | 2.7 | 96.7 | - |
| A. citriarbusti (now A. alternata) | CBS 102598 | Alternaria | Ion Torrent | 34.8 | $38 \times$ | 1.7 | 98.1 | 1.4 |
| A. gaisen | CBS 118488 | Alternaria | Illumina | 35.2 | $182 \times$ | 1.8 | 96.7 | 2.8 |
| A. tenuissima (now A. alternata) | CBS 918.96 | Alternaria | Illumina | 33.5 | $260 \times$ | 1.4 | 98.2 | 1.5 |
| A. alternantherae | CBS 124392 | Alternantherae | Illumina | 35.0 | $210 \times$ | 16.5 | 89.3 | 8.0 |
| A. solani | CBS 109157 | Porri | Ion Torrent | 32.6 | 50× | 1.5 | 87.9 | 9.0 |
| A. avenicola | CBS 121459 | Panax | Illumina | 39.1 | $200 \times$ | 11.9 | 87.2 | 9.5 |
| A. infectoria | CBS 210.86 | Infectoriae | Illumina | 36.5 | $200 \times$ | 5.3 | 85.1 | 10.3 |
| A. papaveraceae | CBS 116607 | Crivellia | Illumina | 33.8 | $220 \times$ | 5.3 | 85.8 | 10.3 |
| A. brassicicola ${ }^{\text {l }}$ | ATCC $96836=$ CBS 118699 | Brassicicola | - | 32.0 | - | 7.1 | 86.6 | - |

Table 3. Assembly statistics of the Alternaria transcriptome profiles.

| Species | Strain number | Section | \% SNP ${ }^{2}$ |
| :--- | :--- | :--- | :---: |
| A. alternata | CBS $916.96^{1}$ | Alternaria | 0.0 |
| A. arborescens | CBS 102605 | Alternaria | 1.8 |
| A. citriarbusti (now A. alternata) | CBS 102598 | Alternaria | 1.0 |
| A. citricancri (now A. alternata) | CBS 119543 | Alternaria | 0.9 |
| A. gaisen | CBS 118488 | Alternaria | 1.8 |
| A. mali (now A. alternata) | CBS 106.24 | Alternaria | 0.9 |
| A. tenuissima (now A. alternata) | CBS 918.96 | Alternaria | 0.8 |
| A. tomaticola (now A. alternata) | CBS 118814 | Alternaria | 0.9 |
| A. toxicogenica (now A. alternata) | CBS 102600 | Alternaria | 0.9 |
| A. alternantherae | CBS 124392 | Alternantherae | 6.1 |
| A. infectoria | CBS 210.86 | Infectoriae | 8.5 |
| A. papaveraceae | CBS 116607 | Crivellia | 8.4 |

${ }^{1}$ Reference isolate.
${ }^{2}$ SNPs / covered base ( $>10 \times$ ), duplicates removed.
using BWA (Li \& Durbin 2009) using the BWA-MEM algorithm (v. 0.7.5a-r405). Transcript reads were trimmed prior to mapping using fastx-tools. Duplicated reads were identified and marked using Picard tools (http://broadinstitute.github.io/picard). Using GATK, transcript reads were splitted into exons and overhangs were removed. Subsequently, transcript and genomic reads were locally realigned to minimise the number of mismatches over all reads. Afterwards, genomic variants (SNPs) were called using GATK's UnifiedGenotyper (standard call and emitting threshold of 20 ; haploid organisms), and the resulting SNPs were filtered based on quality ( $\mathrm{Qual}=50$ ), depth $(\mathrm{DP}=10)$ and allelic frequency $(\mathrm{AF}=0.9)$.

Conserved eukaryotic orthologous group (KOG) proteins were identified using the Core Eukaryotic Genes Mapping Approach (CEGMA) pipeline (Parra et al. 2007). For the conservation table we focused on the five available genomes of section Alternaria to avoid alignment problems that could affect the conservation values.

The reference sequence alignment-based phylogeny builder (REALPHY) v. 1.09 (Bertels et al. 2014) was used to construct a phylogenetic tree based on the whole-genome and transcriptome reads and the previously assembled Alternaria genomes. Briefly, short reads (genome and transcriptome) as well as short sequence fragments ( 100 nt ) derived from the previously assembled genomes were mapped against the reference genome (A. alternata CBS 916.96) using Bowtie2. Subsequently, polymorphic as well as non-polymorphic sites were filtered (per base quality [20], coverage [10] and polymorphism frequency [0.95]) and extracted. Only sites that were present in all species were retained. The derived pseudo-molecule was used to infer a maximum likelihood phylogenetic tree using PhyML using the generalised time reversible (GTR) nucleotide substitution model. The robustness of the phylogeny was assessed by 1000 bootstrap replicates.

## PCR and sequencing

DNA extraction for gene sequencing was performed using the UltraClean ${ }^{\mathrm{TM}}$ Microbial DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's
instructions. The SSU, LSU, ITS, GAPDH, RPB2 and the TEF1 gene regions were amplified and sequenced as described in Chapter 2 and the Alt a 1 gene as described in Chapter 4. The endoPG and OPA10-2 gene regions were amplified using the primers PG3 and PG2b and OPA10-2L and OPA10-2R (Andrew et al. 2009). For the KOG1058 and KOG1077 gene regions the primers KOG1058F2 ( $5^{\prime}$-GAG TCA CGT TAY CGC ASC-3') and KOG1058R2 ( $5^{\prime}$-TGG CTK ACG GAR ACG-3') and KOG1077F2 ( $5^{\prime}$-GGA GCA GTC GGG CAA CG-3’) and KOG1077R2 (5'-ATT CRT GTT GTA CRA TCG C-3') were designed from the genomic data. The PCRs were performed in an Applied Biosystems ${ }^{\circledR} 2720$ Thermal Cycler (Thermo Fisher Scientific), in a total volume of $12.5 \mu$ l. The PCR mixtures consisted of $1 \mu 1$ genomic DNA, $1 \times \mathrm{NH}_{4}$ reaction buffer (Bioline, Luckenwalde, Germany), 2 mM (endoPG, OPA10-2) or $1.6 \mathrm{mM} \mathrm{MgCl}{ }_{2}$ (KOG1058, KOG1077), $20 \mu \mathrm{M}$ of each dNTP, $0.2 \mu \mathrm{M}$ of each primer and 0.5 U Taq DNA polymerase (Bioline). The PCR conditions consisted of an initial denaturation step of 5 min at $94^{\circ} \mathrm{C}$ followed by 40 cycles of 30 s at $94^{\circ} \mathrm{C}, 30 \mathrm{~s}$ at $50^{\circ} \mathrm{C}$ and 30 s at $72^{\circ} \mathrm{C}$ for endoPG, 35 cycles of 30 s at $94^{\circ} \mathrm{C}, 30 \mathrm{~s}$ at $62^{\circ} \mathrm{C}$ and 45 s at $72^{\circ} \mathrm{C}$ for OPA10-2, and 35 cycles of 30 s at $94{ }^{\circ} \mathrm{C}, 30 \mathrm{~s}$ at $59^{\circ} \mathrm{C}$ and 60 s at $72^{\circ} \mathrm{C}$ for KOG1058 and KOG1077, and a final elongation step of 7 min at $72{ }^{\circ} \mathrm{C}$. The PCR products were sequenced in both directions using the PCR primers and a BigDye ${ }^{\circledR}$ Terminator v. 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific), and analyzed with an ABI Prism 3730XL DNA Analyzer (Thermo Fisher Scientific) according to the manufacturer's instructions. Consensus sequences were computed from forward and reverse sequences using the BioNumerics v. 4.61 software package (Applied Maths, St-Martens-Latem, Belgium). All generated sequences were deposited in GenBank (Table 1).

## Phylogenetic analyses

Multiple sequence alignments of individual data partitions were generated with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html), and manually adjusted. The best nucleotide substitution model for each partition was determined with Findmodel (http:// www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). For the ITS and OPA102 partitions a K80 model with a gamma-distributed rate variation was suggested, for the SSU, LSU, TEF1 and Alt a 1 partitions a HKY model, with gamma-distributed rate variation for LSU and Alt a 1, for the GAPDH, RPB2 and KOG1077 partitions a TrN model with gamma-distributed rate variation and for the endoPG and KOG1058 partitions a GTR model with gamma-distributed rate variation. Bayesian analyses were performed with MrBayes v. 3.1.2 (Huelsenbeck \& Ronquist 2001, Ronquist \& Huelsenbeck 2003) on the individual data partitions as well as the combined aligned dataset. The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The sample frequency was set at 500 for the combined analysis and the less informative loci (SSU, LSU, ITS and TEF1) and at 100 for the remaining loci. The temperature value of the heated chain was 0.1 and the run stopped when the average standard deviation of split frequencies fell below 0.01 . Burn-in was set to $25 \%$ after which the likelihood values were stationary. Tracer v. 1.5.0 (Rambaut \& Drummond 2009) was used to confirm the convergence of chains. A maximum-likelihood analysis including 500 bootstrap replicates using RAxML v. 7.2.6 (Stamatakis \& Alachiotis 2010) was additionally run on the combined aligned dataset. Sequences of $A$. alternantherae (CBS 124392) were used as outgroup. The resulting trees were printed with TreeView v. 1.6.6 (Page 1996) and, together with the alignments, deposited into TreeBASE (http://www. treebase.org).

## Species recognition and naming in Alternaria section Alternaria

Individual gene trees were generated as described in the 'Phylogenetic analyses' part above and examined manually. A species clade was only recognised as unique if it was well-supported and monophyletic with all of its included isolates in multiple single-gene phylogenies, and no incongruencies were observed in the other single-gene phylogenies, e.g. the included isolates clustered together in all single-gene phylogenies. Unique molecular markers for the recognised species, which separates them from the other species in section Alternaria, are described with the species below. Unique fixed nucleotide positions were derived from the respective alignments of the separate loci deposited in TreeBASE based on a comparison of the sequences of all isolates from the specific species to the sequences of all isolates of the other recognised species within section Alternaria.

We further propose to standardise the taxonomic terms used and favour the use of the trinomial system introduced by Rotem (1994). When differences in host affinity are observed within the isolates of one (of the above-defined) species, the third epithet, the forma specialis, defines the affinity to this specific host in accordance with the produced toxin causing this affinity. When different toxins are produced on the same host, but these toxins affect different host species, the term pathotype will be used in addition. All isolates which are not confined to specific hosts and / or toxins should retain only the binomial name until such specificity is found. For examples, please refer to the species notes under $A$. alternata below and the Discussion.

## RESULTS

## NGS

We sequenced nine Alternaria species using Ion Torrent ${ }^{\mathrm{TM}}$ or Illumina ${ }^{\circledR}$ sequencing technologies, yielding between $38 \times$ and $>260 \times$ average genome coverage (Table 2). The assembled genomes ranged in size from $33.3-35.2 \mathrm{Mb}$ within section Alternaria and from $32.0-39.1 \mathrm{Mb}$ for all Alternaria genomes (Table 2). To characterise the assembled genomes, we identified and classified the repetitive complement of each individual genome using a combination of $d e$ novo prediction and identification of known repetitive elements. Surprisingly, the number of repetitive sequences differed significantly between different Alternaria genomes. Within section Alternaria, the number of repetitive sequences is comparably low; only $1.4-2.7 \%$ of each genome was classified as repetitive (Table 2). In contrast, A. avenicola and A. alternantherae carry significantly higher percentages of repetitive elements, $>10 \%$ and $>15 \%$, respectively (Table 2).

To assess the genomic differences between the included species, we performed whole-genome alignments to the reference genome of $A$. alternata (CBS 916.96). These alignments revealed 96.7-98.2 \% genome identity within section Alternaria compared to $85.1-89.3$ \% genome identity between isolates from other sections with $A$. alternata. Furthermore, we assessed the number of single nucleotide polymorphisms (SNPs) between the different species by mapping genomic reads to the reference genome of $A$. alternata (CBS 916.96). We observed 1.4-2.8 \% SNPs between isolates from section Alternaria, while the percentage of SNPs found in isolates from different sections was considerably higher, ranging from 8.0-10.3 \% (Table 2).

To further characterise the genus, we derived deep transcriptome sequences of 12 isolates that were mapped to the reference isolate of A. alternata (CBS 916.96). In this case, we observed


Fig. 1. PhyML tree based on the whole-genome and transcriptome reads of 15 Alternaria species using REALPHY. The bootstrap support values are given at the nodes; thickened lines indicate a fully supported node. The grey box represents species which are now synonymised under $A$. alternata. The tree was rooted to A. papaveraceae (CBS 116607).
$0.8-1.8 \%$ SNPs among the isolates from section Alternaria, while the isolates from other sections displayed between 6.1 and $8.5 \%$ SNPs (Table 3).

We identified marker genes with potential discriminatory power by predicting a set of conserved eukaryotic genes (KOG) in the genomes of the five assembled section Alternaria genomes using the CEGMA pipeline. Out of 380 included KOGs, 326 ( $86 \%$ ) had a conservation level of $\geq 98 \%$. Therefore, we focused on the 25 KOGs with the lowest degree of conservation, ranging from 83.0-97.3 \%, and evaluated their discriminatory power. KOGs that were not able to distinguish all morphospecies included in the whole-genome and transcriptome sequencing were immediately rejected. We eventually designed primers spanning the first 5 introns of KOG1058 and KOG1077 (see the 'PCR and sequencing' part of the 'Material and Methods'). These proteins were found on place 16 and 23 in the conservation table and both act in the vesicle coat complex, although in different systems; namely COPI versus AP-2.

The pseudo-molecule derived from the whole-genome and transcriptome reads with REALPHY contained 1750944 nt . The topology from the REALPHY phylogeny (Fig. 1) corresponds to the multi-gene phylogeny based on a five-gene combined dataset (Fig. 3 in Lawrence et al. 2013) and a three-gene combined dataset (Fig. 1 in Chapter 2). Section Alternantherae and section Porri are the sister sections of section Alternaria, while section Infectoriae and section Crivellia, are the most distant sections (Fig. 1).


## Gene-based phylogeny and identification

From the 168 isolates included in the multi-gene phylogeny, the amplification and / or sequencing of two isolates for the RPB2 gene, three for the Alt a 1 gene, one for the endoPG gene and four for the OPA10-2 regions failed (Table 1); these genes were included as missing data in the combined analysis. The aligned sequences of the SSU (1021 aligned characters), LSU (849 aligned characters), ITS (523 aligned characters), GAPDH (579 aligned characters), TEF1 (241 aligned characters), RPB2 (753 aligned characters), Alt a 1 (473 aligned characters), endoPG (448 aligned characters) and OPA10-2 (634 aligned characters) gene regions contained $6,9,27,60,42,87,110,59$ and 123 unique site patterns, respectively. Because of the low informative value of the SSU and LSU sequences ( 6 / 9 unique site patterns out of $1021 / 849$ aligned characters) these genes were excluded from the multi-gene phylogeny. The multi-gene phylogeny based on the remaining seven gene regions contained 3651 characters including alignment gaps, which, after discarding the burn-in phase, resulted in a $50 \%$ majority rule consensus tree based on 15002 trees from two runs (Fig. 2).

The alignments of the additional gene regions that were sequenced, KOG1058 and KOG1077, consisted of 921 and 781 aligned characters, respectively, of which 118 and 78 were unique site patterns. The amplification and / or sequencing of the KOG1077 gene failed in six of the 49 isolates, representing the species A. alstroemeriae, A. iridiaustralis and A. jacinthicola (Table 1). Since the KOG1077 sequences could not separate A. longipes from A. gossypina, we did not put any further effort in optimising the primers to obtain the missing data.

Although the single-gene phylogenies are not fully congruent in terms of species resolution (see TreeBASE), 11 clades can be distinguished consistently within the single-gene phylogenies and in the multi-gene phylogeny (Fig. 2). Eight of those are single species clades representing $A$. alstroemeriae, A. betae-kenyensis, A. eichhorniae, A. gaisen, A. iridiaustralis, A. jacinthicola, A. longipes, and $A$. tomato. Three further clades constitute numerous morphospecies, which we synonymise here under $A$. burnsii, A. gossypina and the $A$. arborescens species complex (AASC). However, the majority of the isolates ( $105 / 168$ ), representing 35 morphospecies, do not form clear phylogenetic clades. The subclades that are formed by these isolates are incongruent between the different gene regions sequenced; no two genes show the same groupings from any of the 100 plus isolates. These morphospecies are synonymised below under $A$. alternata.

None of the genes sequenced in this study enabled us to distinguish all of the species recognised here on its own (Table 4). The commonly used GAPDH sequence can distinguish all species, except the AASC, from A. alternata. Five genes, namely RPB2, OPA10-2, Alt a 1, endoPG and KOG1058, can separate all species from A. alternata, but fail to separate different pairs of other species from one another (see Table 4). The SSU, LSU and ITS genes were least successful in separating the species accepted in this study. The unique fixed nucleotides per gene region are provided below under the treatment of each species.

Fig. 2. Bayesian 50 \% majority rule consensus tree based on the ITS, GAPDH, TEF1, RPB2, Alt a 1, endoPG and OPA10-2 sequences of 168 Alternaria strains. The Bayesian posterior probabilities $>0.75$ $(\mathrm{PP})$ and RAxML bootstrap support values $>65(\mathrm{ML})$ are given at the nodes $(\mathrm{PP} / \mathrm{ML})$. Thickened lines indicate a PP of 1.0 and ML of 100 . Species names between parentheses represent synonymised species names. Ex-type strains are indicated with T and representative strains with R. The ex-type strains of here recognised species are printed in bold face. The tree was rooted to $A$. alternantherae (CBS 124392).

Table 4. Comparison of gene ability to distinguish species in section Alternaria.


## Species in section Alternaria

Alternaria alstroemeriae E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 444. 2007.

Specimens examined: Australia, from leaf of Alstroemeria sp. (Alstroemeriaceae), Jul. 2005, C.F. Hill, culture ex-type CBS 118809 = E.G.S. 52.068. USA, California, Sacramento, from leaf spot of Alstroemeria sp., before Apr. 2002, D. Fogle, CBS 118808 = E.G.S. 50.116.

Unique fixed nucleotides: GAPDH position 485 (T); RPB2 position 162 (G); TEF1 position 52 (C), 143 (C), 165 (T), 205 (G); OPA10-2 position 120 (T), 151 (T), 303 (G), 318 (G), 330 (C), 390 (G), 417 (C), 486 (G); Alt a 1 position 157 (T), 178 (T), 404 (A); endoPG position 37 (A), 46 (C), 316 (T); KOG1058 position 51 (C), 514 (T), 533 (C).

Alternaria alternata (Fr.) Keissl., Beih. Bot. Centralbl., Abt. 2, 29: 434. 1912.
Basionym: Torula alternata Fr., Syst. Mycol. (Lundae) 3: 500. 1832. (nom. sanct.)
= Alternaria tenuis Nees, Syst. Pilze (Würzburg): 72. 1816 [1816-1817].
$=$ Helminthosporium tenuissimum Kunze ex Nees \& T. Nees, Nova Acta Acad. Caes. Leop.-
Carol. German. Nat. Cur. 9: 242. 1818.
$\equiv$ Macrosporium tenuissimum (Nees \& T. Nees) Fr., Syst. Mycol. 3: 374. 1832. (nom. sanct.)
$\equiv$ Clasterosporium tenuissimum (Nees \& T. Nees: Fr.) Sacc., Sylloge Fungorum (Abellini) 4: 393. 1886.
$\equiv$ Alternaria tenuissima (Nees \& T. Nees: Fr.) Wiltshire, Trans. Brit. Mycol. Soc. 18: 157. 1933.
$=$ Macrosporium fasciculatum Cooke \& Ellis, Grevillea 6: 6.1877.
$\equiv$ Alternaria fasciculata (Cooke \& Ellis) 1.R. Jones \& Grout, Bull. Torrey Bot. Club 24: 257. 1897.
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$\equiv$ Alternaria caudata (Cooke \& Ellis) E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 496. 2007.
$=$ Macrosporium maydis Cooke \& Ellis, Grevillea 6: 87. 1878.
$=$ Macrosporium inquinans Cooke \& Ellis, Grevillea 7: 39. 1878
= Macrosporium meliloti Peck, Rep. (Annual) NewYork State Mus. Nat. Hist. 33: 28. 1880.
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= Alternaria rhadina E.G. Simmons, Mycotaxon 48: 101. 1993.
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= Alternaria broussonetiae T.Y. Zhang, W.Q. Chen \& M.X. Gao, Mycotaxon 72: 439. 1999.
= Alternaria citriarbusti E.G. Simmons, Mycotaxon 70: 287. 1999.
= Alternaria citrimacularis E.G. Simmons, Mycotaxon 70: 277. 1999.
= Alternaria dumosa E.G. Simmons, Mycotaxon 70: 310. 1999.
= Alternaria interrupta E.G. Simmons, Mycotaxon 70: 306. 1999.
= Alternaria limoniasperae E.G. Simmons, Mycotaxon 70: 272. 1999.
= Alternaria perangusta E.G. Simmons, Mycotaxon 70: 303. 1999.
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= Alternaria toxicogenica E.G. Simmons, Mycotaxon 70: 294. 1999.
= Alternaria turkisafria E.G. Simmons, Mycotaxon 70: 290. 1999.
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= Alternaria astragali Wangeline \& E.G. Simmons, Mycotaxon 99: 84. 2007.
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= Alternaria daucifolii E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 518. 2007.
= Alternaria herbiphorbicola E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 608. 2007.
= Alternaria pulvinifungicola E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 514. 2007.
= Alternaria postmessia E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 598. 2007.
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= Alternaria soliaegyptiaca E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 506. 2007.
$=$ Alternaria tomaticola E.G. Simmons \& Chellemi, CBS Biodiversity Ser. (Utrecht) 6: 528. 2007.
= Alternaria vaccinii E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 432. 2007.
= Alternaria viniferae Yong Wang bis, Y.Y. Than, K.D. Hyde, X.H. Li, Mycol. Progr. 13: 1124. 2014.

Type and representative specimens examined: Canada, Manitoba, from Euphorbia esula (Euphorbiaceae), 1982, K. Mortensen, culture ex-type of A. angustiovoidea CBS 195.86 = E.G.S. 36.172 = DAOM 185214. China, Hebei, from fruit of Pyrus bretschneideri (Rosaceae), 2001, R.G. Roberts, culture ex-type of $A$. yali-inficiens CBS 121547 = E.G.S. 50.048; Shaanxi, Hanzhong, from Platycodon grandiflorus (Campanulaceae), before Dec. 2001, T.Y. Zhang, culture ex-type of A. platycodonis CBS 121348 = E.G.S. 50.070; Shangdong, Changqing, from Broussonetia papyrifera (Moraceae), 13 Sep. 1996, T.Y. Zhang, culture ex-type of $A$. broussonetiae CBS 121455 = E.G.S. 50.078; Shangdong, Jinan, from Sanguisorba officinalis (Rosaceae), 19 Sep. 1996, M.X. Gao, culture ex-type of $A$. sanguisorbae CBS 121456 = E.G.S. 50.080. Denmark, Sjaelland, Clausdal, from Godetia sp. (Onagraceae), 27 Jul. 1942, P. Neergaard, culture ex-type of $A$. godetiae CBS $117.44=$ E.G.S. $06.190=$ VKM F-1870. Egypt, Sabet, from soil, before Jan. 1933, culture ex-type of $A$. soliaegyptiaca CBS $103.33=$ E.G.S. $35.182=$ IHEM 3319. India, from Arachis hypogaea (Fabaceae), 1 Dec. 1980, L.V. Gangawane, culture ex-epitype CBS $916.96=$ CBS $110977=$ CBS $115616=$ E.G.S. $34.016=$ IMI 254138. Israel, from Minneola tangelo (Rutaceae), before Nov. 1996, Z. Solel, culture ex-type of $A$. interrupta CBS $102603=$ E.G.S. 45.011; Mayan Zvi, from Minneola tangelo, before Nov. 1996, Z. Solel, culture ex-type of A. dumosa CBS 102604 = E.G.S. 45.007. Japan, from fruit of Citrus unshiu (Rutaceae), 1968, K. Tubaki, culture ex-type of A. pellucida CBS $479.90=$ E.G.S. 29.028; from leaf of Pyrus pyrifolia (Rosaceae), 1990, K. Nagano, culture ex-type of A. rhadina CBS 595.93. Turkey, Kuzucuoglu, from Minneola tangelo, May 1996, Y. Canihos, culture ex-type of A. turkisafria CBS $102599=$ E.G.S. 44.166; Adana region, from Minneola tangelo, May 1996, Y. Canihos, culture ex-type of A. perangusta CBS 102602 = E.G.S. 44.160 . UK, from Dianthus chinensis (Caryophyllaceae), 20 Feb. 1981, A.S. Taylor, representative isolate of A. tenuissima CBS $918.96=$ E.G.S. 34.015 = IMI 255532. USA, from Malus sylvestris (Rosaceae), before Dec. 1924, J.W. Roberts, culture ex-type of A. mali CBS $106.24=$ E.G.S. $38.029=$ ATCC 13963; Arizona, Yuma, from Brassica oleracea (Brassicaceae), Apr. 1982, R.H. Morrison, culture ex-type of A. brassicinae CBS 118811 = E.G.S. 35.158; California, from fruit of Citrus sinensis (Rutaceae), before Nov. 1947, D.E.

Bliss, representative isolate of $A$. citri CBS 102.47 = E.G.S. 02.062; California, Los Angeles, from Citrus paradisi (Rutaceae), 12 Jul. 1947, L. Davis, culture ex-type of A. citricancri CBS 119543 = E.G.S. 12.160; Colorado, from leaf of Allium sp. (Alliaceae), F.A. Weiss, culture ex-epitype of A. palandui CBS $121336=$ E.G.S. $37.005=$ ATCC 11680; Colorado, Fort Collins, from the root of Stanleya pinnata (Brassicaceae), 19 Jun. 2002, A. Wangeline, culture ex-type of A. seleniiphila CBS 127671 = E.G.S. 52.121; Florida, Lake Alfred, from leaf lesion of Citrus jambhiri (Rutaceae), before Jul. 1997, culture ex-type of A. limoniasperae CBS 102595 = E.G.S. 45.100; Florida, Lake Alfred, from leaf lesion of Citrus jambhiri, before Jul. 1997, culture ex-type of A. citrimacularis CBS 102596 = E.G.S. 45.090 ; Florida, Lake Alfred, from leaf spot of Minneola tangelo, before Feb. 1998, culture ex-type of $A$. citriarbusti CBS 102598 = E.G.S. 46.141; Florida, Lake Alfred, from Minneola tangelo, 19 Dec. 1980, J.O. Whiteside, culture ex-type of A. postmessia CBS 119399 = E.G.S. 39.189; Florida, Quincy, from Solanum lycopersicum (Solanaceae), June 1996, D. Chellemi, culture ex-type of $A$. tomaticola CBS 118814 = E.G.S. 44.048; Florida, Wauchula, from Citrus reticulata (Rutaceae), 6 Jun. 1975, J.O. Whiteside, culture ex-type of $A$. toxicogenica CBS $102600=$ E.G.S. $39.181=$ ATCC 38963; Florida, Zellwood, from Daucus carota (Apiaceae), Jan 1984, R.H. Morrison, culture ex-type of A. daucifolii CBS 118812 = E.G.S. 37.050; Iowa, from Quercus sp. (Fagaceae), 28 Jul. 1953, A. Engelhard, culture ex-type of A. pulvinifungicola CBS 194.86 = E.G.S. $04.090=$ QM 1347; Maryland, from Euphorbia esula, before Dec. 1991, culture ex-type of $A$. herbiphorbicola CBS $119408=$ E.G.S. 40.140; Massachusetts, Hadley, from fruit of Cucumis sativus (Cucurbitaceae), 24 Sep. 1984, E.G. Simmons, representative isolate of A. caudata CBS 121544 = E.G.S. 38.022; Massachusetts, Rochester, from Cuscuta gronovii (Convolvulaceae), Aug. 1997, F. Caruso, culture ex-type isolate of A. destruens CBS 121454 = E.G.S. 46.069; New Jersey, from Vaccinium sp. (Ericaceae), Oct. 1973, R.A. Cappellini, culture ex-type of $A$. vaccinii CBS $118818=$ E.G.S. 31.032 ; Wyoming, Laramie, from the root of Astragalus bisulcatus (Fabaceae), 8 Jun. 2002, A. Wangeline, culture ex-type of A. astragali CBS 127672 = E.G.S. 52.122. Unknown, from Linum usitatissimum (Linaceae), before Jul. 1934, P.K. Dey, culture ex-type of $A$. lini CBS $106.34=$ E.G.S. $06.198=$ DSM $62019=$ MUCL 10030.

Notes: Both the names Torula alternata and Macrosporium tenuissimum represent sanctioned names by Fries (1832), with the basionym of tenuissimum (1818) being the older. However, we choose to retain the well-established name of the type species of Alternaria, A. alternata above the older name $A$. tenuissima, as this would result in confusion among the user community, and be counterproductive. A proposal to conserve A. alternata over A. tenuissima will be compiled for submission to the Nomenclature Committee of Fungi. The isolate CBS 447.86, isolated from Malva sp. in Marocco, was stored in the CBS collection as Alternaria malvae. The original description of $A$. malvae was from leaf lesions of Malva crispa, from Seine-Inférieure (now called Seine-Maritime), France. Therefore we did not synonymise $A$. malvae under A. alternata. The isolate CBS 106.34, send to the CBS by Dey in 1934 together with a reprint of his paper describing $A$. lini, is recognised as an ex-type isolate. We therefore did synonymise $A$. lini under A. alternata. The very recently described $A$. viniferae is synonymised based on the published GAPDH and Alt a 1 sequences, which cluster within A. alternata. Because of the relative high sequence variability amongst the $A$. alternata isolates, we did not assign unique fixed nucleotides to $A$. alternata. Three formae speciales of $A$. alternata are currently recognised; $A$. alternata $f$. sp. mali for isolates producing the AM-toxin, $f$. $s p$. fragariae for isolates producing the AF-toxin, and $f$. sp. citri with two pathotypes, i.e. $f$. $s p$. citri pathotype rough lemon for isolates producing the ACR-toxin, and $f$. sp. citri pathotype tangerine for isolates producing the ACT-toxin.


Fig. 3. Alternaria burnsii conidia and conidiophores. A-B. CBS 108.27. C-D. CBS 879.95. E-F. CBS 118816. G-H. CBS 118817. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria betae-kenyensis E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 530. 2007.
Specimen examined: Kenya, from Beta vulgaris var. cicla (Chenopodiaceae), before Jun. 2001, ex-type CBS $118810=$ E.G.S. $49.159=$ IMI 385709.

Unique fixed nucleotides: ITS position 464 (C); GAPDH position 28 (C), 55 (A), 512 (T); RPB2 position 204 (T), 363 (T), 369 (G), 447 (G), 468 (T), 480 (A), 507 (A), 627 (G); TEF1 position 213 (G), 218 (C); OPA10-2 position 63 (C), 177 (A), 199 (G), 276 (T), 309 (T), 534 (C), 567 (A), 591 (A); Alt a 1 position 55 (A), 155 (A), 311 (G), 338 (T), 359 (C), 365 (C), 379 (C), $440(\mathrm{~T}), 473(\mathrm{~A})$; endoPG position $10(\mathrm{~T}), 286(\mathrm{~T}), 295(\mathrm{~T}), 372(\mathrm{G})$; KOG1058 position 156 (C), 522 (T), 869 (G); KOG1077 position 121 (A), 178 (C), 373 (A), 402 (C), 763 (C).

Alternaria burnsii Uppal, Patel \& Kamat, Indian J. Agric. Sci. 8: 49. 1938. Fig. 3.
= Alternaria tinosporae E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 508. 2007.
= Alternaria rhizophorae E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 510. 2007.

Specimens examined: India, from Cuminum cyminum (Apiaceae), before Dec. 1938, B.N. Uppal, culture ex-type of A. burnsii CBS 107.38; Saznakhali, from infected leaf of Rhizophora mucronata (Rhizophoraceae), 14 Mar. 1995, ex-type of A. rhizophorae CBS 118816 = E.G.S. 43.145 = IMI 368045; Punjab, from Tinospora cordifolia (Menispermaceae), before Sept. 1987, culture ex-type of $A$. tinosporae CBS $118817=$ E.G.S. $39.14=$ IMI 318433; from human
sputum, Anuradha, CBS 130264. Mozambique, from stem of Gossypium sp. (Malvaceae), Aug. 1950, Quintanilha, CBS 110.50. UK, from Sorghum sp. (Poaceae), 19 Dec. 1985, M. Kalicz, CBS 879.95 = IMI 300779. Unknown, from Gomphrena globosa (Amaranthaceae), before Mar. 1927, K. Togashi, CBS 108.27.

Unique fixed nucleotides: endoPG position 196 (C), 199 (A).
Notes: Although A. burnsii only has two unique fixed nucleotides, the species can molecularly easily be distinguished from $A$. alternata. The low number of unique fixed nucleotides is due to its close phylogenetic relationship to $A$. tomato and $A$. jacinthicola. Most of the nucleotide differences present between $A$. burnsii and the $A$. alternata isolates are also present in the $A$. tomato and / or A. jacinthicola isolates.

Alternaria eichhorniae Nag Raj \& Ponnappa, Trans. Brit. Mycol. Soc. 55: 124. 1970.
Specimens examined: India, Karnataka, Bangalore, from leaf of Eichhornia crassipes (Pontederiaceae), 28 Feb 1966, R. Charudattan, culture ex-type CBS 489.92 = ATCC 22255 = ATCC 46777 = ATCC 201659 = IMI 121518. Indonesia, from leaf of Eichhornia crassipes, before Dec. 1996, representative culture CBS $119778=$ E.G.S. $45.026=$ IMI 372968.

Unique fixed nucleotides: ITS position 105 (T); GAPDH position $36(\mathrm{G}), 162(\mathrm{G}), 168(\mathrm{~T}), 509$ (A); RPB2 position $6(\mathrm{~T}), 549(\mathrm{G})$; TEF1 position $12(\mathrm{C}), 31(\mathrm{G}), 223$ (G); OPA10-2 position 123 (G), 366 (C), 387 (A), 582 (T), 600 (A); Alt a 1 position 67 (T), 130 (A), 298 (A), 356 (A), 397 (C); endoPG position 29 (A), 68 (C), 79 (T), $130(\mathrm{~A}), 148$ (T), 152 (A), 173 (A), 316 (G), 369 (C), 376 (C), 378 (T); KOG1058 position 16 (C), 64 (T), 254 (C), 268 (T), 269 (G), 270 (G), 278 (G), 298 (C), 536 (C), 694 (G), 711 (C); KOG1077 position 62 (T), 162 (C), 166 (C), 189 (C), 195 (C), 234 (G), 235 (C), 348 (C), 350 (C), 564 (A), 685 (A), 715 (A), 776 (T).

Alternaria gaisen Nagano ex Hara, Sakumotsu Byorigaku, Edn 4: 263. 1928.
= Alternaria gaisen Nagano, J. Jap. Soc. Hort. Sci. 32: 16-19. 1920. (nom. illegit., Art. 39.1)
= Alternaria kikuchiana S. Tanaka, Mem. Coll. Agric. Kyoto Univ., Phytopathol. Ser. 28: 27. 1933.
= Macrosporium nashi Miura, Flora of Manchuria and East Mongolia, Part III Cryptogams, Fungi: 513. 1928.

Specimens examined: Japan, Tottori, from Pyrus pyrifolia (Rosaceae), Jul. 1990, E.G. Simmons, representative isolate CBS 118488 = E.G.S. 90.0391; Tottori, from Pyrus pyrifolia, 11 Jul. 1990, E.G. Simmons, representative isolate CBS 632.93 = E.G.S. 90.0512 . Netherlands, host unknown, Aug. 2011, S. I. R. Videira, SV01.

Unique fixed nucleotides: GAPDH position 383 (C), 473 (A); RPB2 position 207 (T), 540 (G); TEF1 position 241 (T); Alt a 1 position 1 (A), 13 (T), 97 (A), 339 (T), 345 (G), 413 (C); endoPG position 130 (C), 172 (A), 250 (T), 361 (T); KOG1058 position 707 (G); KOG1077 position 174 (A).

Alternaria gossypina (Thüm.) J.C.F. Hopkins, Trans. Brit. Mycol. Soc. 16: 136. 1931. Fig. 4. Basionym: Macrosporium gossypinum Thüm., Herb. Mycol. Oecon.: no. 513. 1877.


Fig. 4. Alternaria gossypina conidia and conidiophores. A-B. CBS 100.23. C-D. CBS 104.32. E-F. CBS 107.36. G-H. CBS 102597. Scale bars $=10 \mu \mathrm{~m}$.
= Alternaria grisea Szilv., Arch. Hydrobiol. 3: 546. 1936.
= Alternaria colombiana E.G. Simmons, Mycotaxon 70: 298. 1999.
= Alternaria tangelonis E.G. Simmons, Mycotaxon 70: 282. 1999.
Type: (Lectotype, designated in Simmons 2003) USA, South Carolina, Aiken, from stems of dead Gossypinum herbaceum, 1876, H.W. Ravenel, Macrosporium gossypinum BPI 445306.

Specimens examined: Colombia, Chinchiná, from fruit lesion of Minneola tangelo (Rutaceae), before Nov. 1996, B. L. Castro, culture ex-type of A. colombiana CBS 102601 = E.G.S. 45.017. Sumatra, Toba Heath, from soil, before Jun 1936, A. von Szilvinyi, culture ex-type of $A$. grisea CBS 107.36. USA, Florida, from Minneola tangelo, before Aug. 1997, culture ex-type of A. tangelonis CBS 102597 = E.G.S. 45.114. Zimbabwe, from Gossypium sp. (Malvaceae), before Mar. 1932, J.C.F. Hopkins, culture ex-type of A. gossypina CBS 104.32. Unknown, from Malus domestica (Rosaceae), before Jun. 1923, A.S. Horne, CBS 100.23.

Unique fixed nucleotides: OPA10-2 position 172 (T); KOG1058 position 19 (A), 20 (A).
Notes: Although A. gossypina only has three unique fixed nucleotides, the species can molecularly easily be distinguished from $A$. alternata. The low number of unique fixed nucleotides is due to its close phylogenetic relationship to $A$. longipes. Most of the nucleotide differences present between $A$. gossypina and the $A$. alternata isolates are also present in the $A$. longipes isolates.

The isolate of $A$. gossypina deposited to the CBS by J.C.F. Hopkins, CBS 104.32, is recognised as ex-type culture of A. gossypina and the isolate of A. grisea deposited at the CBS by A. von Szilvinyi, CBS 107.36, is recognised as ex-type isolate of $A$. grisea. The isolate CBS 100.23, from Malus domestica, was deposited at the CBS as $A$. grossulariae. The original type description of this species, however, was from Grossularia sp., from Riga, Letland. Therefore we did not synonymise $A$. grossulariae under $A$. gossypina based on this isolate pending the recollection of authentic material of the former species. By synonymising A. grisea, A. colombiana and $A$. tangelonis under A. gossypina, this species now has become an Alternaria species with a broad host range including host species from the Rutaceae, Malvaceae and Rosaceae.

Alternaria iridiaustralis E.G. Simmons, Alcorn \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 434. 2007.

Specimens examined: Australia, Queensland, Brisbane, from Iris sp. (Iridaceae), Oct. 1995, J. Alcorn, culture ex-type CBS 118486 = E.G.S. 43.014; Queensland, Brisbane, from Iris sp., Oct. 1996, J. Alcorn, CBS 118487 = E.G.S. 44.147. New Zealand, Auckland, Grey Lynn, from leaf of Iris sp., 7 Jan. 2001, C.F. Hill, CBS 118404 = E.G.S. 49.078.

Unique fixed nucleotides: ITS position 475 (A); GAPDH position 33 (A), 171 (T), 174 (A), 186 (C), 218 (G), 365 (A); RPB2 position 12 (T), 489 (T), 516 (T), 591 (C); TEF1 position 9 (G), 43 (T), 238 (G); OPA10-2 position 27 (G), 209 (C), 226 (A), 243 (G), 270 (C), 273 (A), 297 (C), 339 (T), 435 (A), 486 (A); Alt a 1 position 28 (T), 73 (C), 97 (G), 109 (T), 111 (G), 224 (A), 256 (T), 266 (A), 267 (G), 350 (G), 361 (A), 388 (C); endoPG position 87 (A), 93 (G), 101 (G), 210 (A), 219 (T), 338 (A), 340 (T), 374 (A); KOG1058 position 25 (C), 48 (A), 498 (C), 569 (T).

Alternaria jacinthicola Dagno \& M.H. Jijakli, J. Yeast Fungal Res. 2: 102. 2011.
= Alternaria capsicicola A. Nasehi, J. Kadir \& F. Abed-Ashtiani, Mycol. Progr. 13: 1044. 2014. (nom. inval., Art. 8.1, Melbourne Code)

Specimens examined: Mali, from leaf of Eichhornia crassipes (Pontederiaceae), 2006, K. Dagno, culture ex-type CBS 133751 = MUCL 53159. Mauritius, from leaf spot of Arachis hypogaea (Fabaceae), 2 Sep. 1959, S. Felix, CBS 878.95 = IMI 77934b. Unknown, from imported fruit of Cucumis melo (Cucurbitaceae) bought in Dutch supermarket, Feb. 2013, U. Damm, UD03.

Unique fixed nucleotides: GAPDH position 479 (A); RPB2 position 6 (T), 549 (G); OPA10-2 position 159 (C); Alt a 1 position 295 (C), 353 (C), 364 (G); endoPG position 19 (T).

Notes: Although $A$. jacinthicola only has a few unique fixed nucleotides, the species can molecularly easily be distinguished from $A$. alternata. The low number of unique fixed nucleotides is due to its close phylogenetic relationship to A. tomato and A. burnsii. Most of the nucleotide differences present between $A$. jacinthicola and the $A$. alternata isolates are also present in the $A$. tomato and / or $A$. burnsii isolates. By including two other isolates with A. jacinthicola, it has become an Alternaria species with a broad host range including species from the Pontederiaceae, Cucurbitaceae and Fabaceae. The recently described A. capsicicola (Nasehi et al. 2014) is synonymised under A. jacinthicola based on its Alt a 1 (KJ508068,

KJ508069) and GAPDH (KJ508064, KJ508065) sequences which are $100 \%$ identical to $A$. jacinthicola. The name $A$. capsicicola is invalid, as two accessions were designated as holotype specimens.

Alternaria longipes (Ellis \& Everh.) E.W. Mason, Mycol. Pap. 2: 19. 1928.
Basionym: Macrosporium longipes Ellis \& Everh., J. Mycol. 7: 134. 1892.
= Alternaria brassicae var. tabaci Preissecker, Fachliche Mitt. Österr. Tabakregie 16: 4. 1916.

Specimens examined: USA, North Carolina, from Nicotiana tabacum (Solanaceae), 1967, E.G. Simmons, CBS 917.96; North Carolina, from Nicotiana tabacum, before Nov. 1971, representative isolate CBS 540.94 = E.G.S. $30.033=$ QM 9589; North Carolina, Colombus County, from Nicotiana tabacum, Aug. 1963, E.G. Simmons, CBS 539.94 = QM 8438; North Carolina, from Nicotiana tabacum, before Nov. 1971, representative isolate CBS $121332=$ E.G.S. 30.048; North Carolina, from Nicotiana tabacum, before Nov. 1971, representative isolate CBS 121333 = E.G.S. 30.051. Unknown, from leaf spot of Nicotiana tabacum, before Oct. 1935, W.B. Tisdale, CBS 113.35.

Unique fixed nucleotides: SSU position 654 (G); ITS position 491 (C); GAPDH position 144 (G); OPA10-2 position 51 (T), 85 (G); KOG1058 position 848 (C).

Note: Although A. longipes only has a few unique fixed nucleotides, the species can molecularly easily be distinguished from $A$. alternata. The low number of unique fixed nucleotides is due to its close phylogenetic relationship to $A$. gossypina. Most of the nucleotide differences present between $A$. longipes and the $A$. alternata isolates are also present in the $A$. gossypina isolates.

Alternaria tomato (Cooke) L.R. Jones, Bull. Torrey Bot. Club 23: 353. 1896.
Basionym: Macrosporium tomato Cooke, Grevillea 12: 32. 1883.
Specimens examined: Unknown, from Solanum lycopersicum (Solanaceae), before Apr. 1930, A.A. Bailey, CBS 103.30; from Solanum lycopersicum, before Mar. 1935, G.F. Weber, CBS 114.35 .

Unique fixed nucleotides: GAPDH position 356 (T); RPB2 position 21 (T), 252 (C), 567 (C); TEF1 position 36 (T); Alt a 1 position 187 (G); KOG1058 position 60 (A), 183 (A); KOG1077 position $588(\mathrm{~T})$.

Notes: Although $A$. tomato only has a few unique fixed nucleotides, the species can molecularly easily be distinguished from $A$. alternata. The low number of unique fixed nucleotides is due to its close phylogenetic relationship to $A$. burnsii and $A$. jacinthicola. Most of the nucleotide differences present between $A$. tomato and the $A$. alternata isolates are also present in the $A$. burnsii and / or A. jacinthicola isolates.

Alternaria arborescens species complex (Fig. 5).
Alternaria arborescens E.G. Simmons, Mycotaxon 70: 356. 1999.
Alternaria cerealis E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 600. 2007.


Fig. 5. Alternaria arborescens species complex conidia and conidiophores. A-B. A. geophila CBS 101.13. C-D. A. arborescens CBS 102605. E-F. A. cerealis CBS 119544. G-H. A. senecionicola CBS 119545. Scale bars $=10 \mu \mathrm{~m}$.

Alternaria geophila Dasz., Bull. Soc. Bot. Genève, 2 Sér. 4: 294. 1912.
Alternaria senecionicola E.G. Simmons \& C.F. Hill, CBS Biodiversity Ser. (Utrecht) 6: 658. 2007.

Type specimens examined: New Zealand, Auckland, Grey Lynn, from blighted Senecio skirrhodon (Compositae), Jul. 2000, C.F. Hill, culture ex-type of A. senecionicola CBS 119545 = E.G.S. 48.130; Auckland, from Avena sativa (Gramineae), Nov. 1995, C.F. Hill, culture extype of A. cerealis CBS $119544=$ E.G.S. 43.072 . Switzerland, from peat soil, before 1913, W. Daszewska, culture ex-type of $A$. geophila CBS 101.13. USA, California, from Solanum lycopersicum (Solanaceae), 23 Apr. 1990, D. Gilchrist, culture ex-type of $A$. arborescens CBS 102605 = E.G.S. 39.128.

Unique fixed nucleotides: RPB2 position 18 (A), 385 (T); TEF1 position 42 (T), 44 (A), 111 (G); OPA10-2 position $330(\mathrm{G}), 504(\mathrm{C})$; Alt a 1 position 333 (T); endoPG position 349 (C); KOG1058 position 625 (C); KOG1077 position 207 (A), 276 (-), 429 (G), 651 (T).

Notes: Although A. geophila is the oldest name in this species complex, we choose to retain the well-known name $A$. arborescens above the relatively unknown name $A$. geophila for the species complex. We were unable to resolve the morphospecies present in this complex with the set of partial gene sequences used in this study and a more detailed study, possibly using
whole-genome sequences of additional isolates from this species complex, is needed. Should this species complex be resolved and $A$. geophila and $A$. arborescens have to be synonymised we strongly suggest priority of the name $A$. arborescens over $A$. geophila. The isolate CBS 126.60 was deposited in the CBS collection as A. maritima; however, the type material of $A$. maritima G.K. Sutherland is unknown, and therefore we did not include $A$. maritima within the AASC pending the recollection of suitable material of $A$. maritima.

## DISCUSSION

To be able to determine whether an isolate should be referred to as forma specialis or pathotype, the species boundaries should first be firmly established. From the seven described pathotypes of Alternaria alternata (Akimitsu et al. 2014), two are now recognised as separate species in section Alternaria, namely $A$. gaisen and $A$. longipes, and one belongs to the $A$. arborescens species complex (AASC). The terms forma specialis (e.g. Neergaard 1945, Joly 1964, Grogan et al. 1975, Vakalounakis 1989, Yoon et al. 1989) and pathotype (Nishimura \& Kohmoto 1983) have both been used to specify the host affinity of strains of $A$. alternata. This affinity to a specific host is in most cases caused by the ability to produce a unique host-specific toxin (HST), which is needed for infection of the specific host. We propose here to standardise the taxonomic terms used according to Rotem's approach (1994). He favoured the use of the trinomial system in which the third epithet, the forma specialis, defines the affinity to a specific host in accordance with the produced toxin. When different toxins are produced on the same host, but these toxins affect different host species, like for instance on Citrus where the ACT- and / or ACR-toxin can be produced by the same $f$. $s p$., which affect tangerine and / or rough lemon, respectively (Masunaka et al. 2005), the term pathotype will be used. The four previously described pathotypes which still reside in A. alternata (Akimitsu et al. 2014), will therefore be named $A$. alternata f. sp. mali for isolates producing the AM-toxin, $f$. sp. fragariae for isolates producing the AF-toxin, $f$. sp. citri pathotype rough lemon for isolates producing the ACR-toxin, and $f$. sp. citri pathotype tangerine for isolates producing the ACT-toxin. All $A$. alternata isolates which are not confined to specific hosts and / or toxins should retain only the binomial name until such specificity is found. Multiple studies showed that HST gene clusters are located on small conditionally dispensable (CD) chromosomes (Tanaka \& Tsuge 2000, Hatta et al. 2002, Akamatsu 2004, Harimoto et al. 2007, 2008, Hu et al. 2012) which can be lost (Johnson et al. 2001) or gained (Salamiah et al. 2001, Masunaka et al. 2005, Akagi et al. 2009), making an isolate either non-pathogenic or pathogenic to the specific host affected by the HST. With the species boundaries set in this study, this loss or gain of a specific gene cluster will not change the binomial part of the species name of an isolate.

Stewart and colleagues (2013a) have suggested that sequence data derived from SCARs would provide sufficient resolution to address lower level phylogenetic hypotheses in Alternaria. The authors developed SCARs from randomly amplified and cloned RAPD-PCR amplicons of which six of the 19 tested on small-spored Alternaria isolates were highly polymorphic. One of them was too variable which made it difficult to align and amplify this region; the remaining five were all more variable then ITS, GAPDH and TEF1, but only one (OPA10-2) showed a higher variability than endoPG. The other four were equally variable as or slightly more variable than endoPG. We have used both endoPG and OPA10-2 in our multi-gene phylogeny, but could only distinguish 11 species of the 52 morphospecies previously described. Also, the molecular phylogenies obtained from our relative low conservative genes based on genome
sequencing, KOG1058 and KOG1077, could not provide sufficient resolution to distinguish the known morphospecies. The incongruencies between the single-gene phylogenies, together with the high similarity found in the sequenced genomes of section Alternaria and the low SNP count derived by the genomic and transcriptomic data between isolates of section Alternaria led us to the conclusion to synonymise 35 Alternaria species under $A$. alternata. As mentioned above, the detection of host-specific toxins could eventually give rise to several new formae speciales of $A$. alternata.

Most of the synonymised species ( $10 / 35$ species) under $A$. alternata were described in 2007 (Simmons), and are only based on a single isolate that was collected long before the year of description (A. brassicinae, A. citricancri, A. herbiphorbicola, A. pulvinifungicola, A. postmessia, A. soliaegyptiaca, A. vaccinii). As far as we know, no new isolates of these species are reported in literature after their original description. Studies on the presence of host-specific toxins in these isolates could show if they should become a new $f$. $s p$. of $A$. alternata. Nine of the synonymised species are described in a paper on the classification of citrus pathogens (Simmons 1999). The validity of all these small-spored species described from citrus was already questioned by a molecular study performed in later years (Peever et al. 2004). The authors already advocated that all small-spored citrus-associated isolates of Alternaria should collapse into a single phylogenetic species, A. alternata. Also the validity of the name $A$. mali, the cause of Alternaria blotch of apple, which occurs on the European quarantine lists, was questioned in recent years (Rotondo et al. 2012, Harteveld et al. 2013). The authors describe the association of multiple Alternaria species-groups with leaf blotch and fruit spot diseases of apple in Italy and Australia respectively, and could not separate the $A$. mali reference isolate from ' $A$. tenuissima' isolates. Based on the approach described in the present study, the only way to distinguish $A$. alternata f. sp. mali, which is of high importance as quarantine organism, is to detect the AM-toxin that gives the name to these isolates (Johnson et al. 2000).

The isolates constituting the AASC show some internal molecular and morphological variation, but can clearly be separated from the A. alternata cluster based on molecular data. Both A. cerealis and A. senecionicola were marked by Simmons (2007) as having an arborescentlike sporulation pattern, but not all isolates from the AASC display this typical arborescent-like sporulation pattern (Fig. 5). This is illustrated by the fact that 12 out of the 28 isolates, which cluster in the AASC, were stored in the CBS collection as either A. alternata or A. tenuissima (Table 1). Because of the inconsistencies in morphology and molecular data in the AASC, more research is needed before conclusions can be drawn on the species present in this complex. Next to the known pathogenicity of $A$. arborescens on tomato, caused by the production of the ALtoxin, studies on Alternaria spp. show that isolates from the AASC can also cause diseases on apple (Rotondo et al. 2012, Harteveld et al. 2013, 2014) and can act as post-harvest pathogens on apple and citrus (Kang et al. 2002, Serdani et al. 2002). The presence of multiple human isolates in the AASC stresses the importance of additional research on this species complex. To our knowledge, $A$. arborescens was not recognised as being of medical importance before. One recent publication (Hu et al. 2014) does describe A. arborescens as the causative agent of a cutaneous Alternariosis in a healthy person, but the identification was based on ITS alone, a locus which cannot distinguish $A$. arborescens from multiple other species now recognised in section Alternaria (Table 4). In the end it might well be that $A$. arborescens needs the same treatment as $A$. alternata, and that it will be divided into different formae speciales based on the specific host they infect, and the toxin gene cluster they exploit.

The genome size ranged from $32.0-39.1 \mathrm{Mb}$ within the Alternaria genomes (Table 2), which can only be partly explained by differences in repeat content between the genomes. The
isolates with the highest repeat content, A. avenicola ( $\sim 12 \%$ repeats) and $A$. alternantherae ( $\sim 16 \%$ repeats), have a relatively large genome size ( 39.1 and 35.0 Mb ), but A. infectoria with a genome size of 36.5 Mb contains only $\sim 5 \%$ of repeats (Table 2). The percentage of repeats within section Alternaria is relatively low, 1.4-2.7 \%, with the highest percentage of repeats in the $A$. arborescens genome. The isolates which we now named $A$. alternata only ranged from $1.4-1.7 \%$. The genome assembly shows a high similarity between the isolates within section Alternaria; 96.7-98.2 \% genome identity within section Alternaria, compared to 85.1-89.3 \% genome identity between isolates from other sections with the reference genome of $A$. alternata (CBS 916.96). This is confirmed by the percentage of SNPs found in the whole-genome and transcriptome reads; 1.4-2.8 and $0.8-1.8 \%$ SNPs in respectively the whole-genome and transcriptome reads between isolates from section Alternaria, compared to $8.0-10.3 \%$ and $6.1-$ $8.5 \%$ SNPs found in isolates from different sections with the $A$. alternata reference genome. The species boundaries proposed here for section Alternaria are corroborated by the percentage of SNPs found in both the genome and transcriptome studies. The species now synonymised under $A$. alternata show 1.4-1.5 \% SNPs in their whole-genome reads compared to $2.8 \%$ in $A$. gaisen and $\leq 1 \%$ of SNPs in their transcriptome reads compared to the reference isolate, while the species retained as separate, $A$. gaisen and $A$. arborescens, both show $1.8 \%$ of SNPs in the transcriptome reads.

The need for this research is stressed by examining recent publications on Alternaria spp. from section Alternaria. Two new Alternaria species, which were published during the writing of this manuscript, are both placed in synonymy under an older species name in this study. Based on molecular comparisons, Alternaria capsicicola (Nasehi et al. 2014) is synonymised under A. jacinthicola, and A. viniferae (Tao et al. 2014) is synonymised under A. alternata. Furthermore, the recent descriptions based on ITS alone of $A$. arborescens as the cause of cutaneous Alternariosis in a healthy person (Hu et al. 2014) and of A. longipes as the cause of a severe leaf spot disease on potato (Shoaib et al. 2014) need to be re-investigated by employing a more robust molecular dataset. As already mentioned above, $A$. arborescens cannot be separated from $A$. alternata based on the ITS region alone, and the 1 unique fixed nucleotide in the ITS sequence which separates $A$. longipes from $A$. alternata is not present in the ITS sequence from the isolate causing the leaf spot in potato. These are most likely not the only examples of species of Alternaria section Alternaria treated in recently published manuscripts which need to be confirmed by or subjected to a multilocus sequence analysis in light of the present study. We hope that the research presented here will make the correct identification of species in section Alternaria easier for other researchers confronted with these species.

## CONCLUSIONS

Based on genome comparisons and molecular phylogenies, Alternaria section Alternaria consists only of 11 phylogenetic species and one species complex. Thirty-five morphospecies, which cannot reliably be distinguished based on the multi-gene phylogeny, are synonymised under $A$. alternata. When a specific HST-gene cluster is demonstrated in an A. alternata isolate, this isolate will be named as a $f$. sp. of $A$. alternata. Currently three formae speciales of $A$. alternata are recognised, of which $f$. sp. citri consists of two pathotypes, according to the host species the HST acts upon. The AASC can be distinguished from all species now recognised within section Alternaria, but the inconsistencies in morphology and molecular data makes further research necessary. By providing guidelines for the naming and identification of species
in Alternaria section Alternaria, we hope to resolve the past confusion in this section. The provided unique fixed nucleotides will help plant pathologists and medical mycologists to choose which genes to sequence for quick and accurate identification of their species of interest.

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# Diversity and movement of indoor Alternaria alternata across the mainland USA 

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#### Abstract

Alternaria spp. from section Alternaria are frequently associated with hypersensitivity pneumonitis, asthma and allergic fungal rhinitis and sinusitis. Since Alternaria is omnipresent in the outdoor environment, it is thought that the indoor spore concentration is mainly influenced by the outdoor spore concentration. However, few studies have investigated indoor Alternaria isolates, or attempted a phylogeographic or population genetic approach to investigate their movement. Therefore, the aim of the current study was to investigate the molecular diversity of indoor Alternaria isolates in the USA, and to test for recombination, using these approaches. Alternaria isolates collected throughout the USA were identified using ITS, GAPDH and endoPG gene sequencing. This was followed by genotyping and population genetic inference of isolates belonging to Alternaria section Alternaria together with 37 reference isolates, using five microsatellite markers. Phylogenetic analyses revealed that species of Alternaria section Alternaria represented $98 \%$ ( 153 isolates) of the indoor isolates collected throughout the USA, of which 137 isolates could be assigned to $A$. alternata, 15 to the $A$. arborescens species complex and a single isolate to $A$. burnsii. The remaining $2 \%$ (3 isolates) represented section Infectoriae (single isolate) and section Pseudoulocladium (2 isolates). Population assignment analyses of the 137 A. alternata isolates suggested that subpopulations did not exist within the sample. The $A$. alternata isolates were thus divided into four artificial subpopulations to represent four quadrants of the USA. Forty-four isolates representing the south-western quadrant displayed the highest level of uniqueness based on private alleles, while the highest level of gene flow was detected between the south-eastern ( 32 isolates) and south-western quadrants. Genotypic diversity was high for all quadrants, and a test for linkage disequilibrium suggested that $A$. alternata has a cryptic sexual cycle. These statistics could be correlated with environmental factors, suggesting that indoor $A$. alternata isolates, although extremely diverse, have a continental distribution and high levels of gene flow over the continent.


## INTRODUCTION

Although environmental Alternaria spp. are not considered as pathogens, their omnipresence is frequently associated with hypersensitivity pneumonitis, asthma and allergic fungal rhinitis and sinusitis in humans (Pastor \& Guarro 2008). Allergic rhinitis is the most common form of noninfectious rhinitis (Randriamanantany et al. 2010), while allergic (extrinsic) asthma is the most common form of asthma, affecting over $50 \%$ of 20 million asthma sufferers (Salo et al. 2006).

The primary dispersal method of species of Alternaria is by the release of conidia (asexual spores) into the air. It has been suggested that changes in temperature and relative air humidity can trigger spore release from plant material (Timmer et al. 1998). The concentration of allergenic airborne spores can thus be linked to the release of spores from infected plants during dry / wet cycles. Additionally, it is possible that the environment contributes to the genetic diversity of populations of airborne Alternaria. For example, areas with large fluctuations in humidity and temperature, and where agricultural activities are prevalent, may be conducive to the generation of diversity that can counteract the selective pressures imposed by the environment. Since Alternaria is omnipresent in the outdoor environment, it is thought that the indoor spore concentration is mainly influenced by the outdoor spore concentration. However, the indoor level of fungal spores in the air is influenced by the activity in the room, fluctuations in temperature and relative humidity, and the ventilation rate (Samson et al. 2010).

Alternaria alternata (belongs to Alternaria section Alternaria) (cf. Lawrence et al. 2013, Chapter 2) is thought to be the main airborne allergen of the genus Alternaria (Horner et al. 1995, Pulimood et al. 2007, Kuna et al. 2011). Alternaria section Alternaria consists of more than 50 pathogenic and non-pathogenic morpho-species (Chapter 2). These morpho-species display very low levels of DNA sequence variation, and are therefore difficult to distinguish at the sequence level (Peever et al. 2004, Andrew et al. 2009). A recent study based on wholegenome sequencing supplemented with transcriptome profiling and multi-gene sequencing only recognized 11 phylogenetic species and one species complex in section Alternaria (Chapter 5). As a result, 35 morpho-species were placed in synonymy with A. alternata. Alternaria alternata is also associated with diseases of citrus, and like other airborne fungi, it displays a worldwide distribution (Stewart et al. 2014). Nonetheless, several studies (e.g. Peever et al. 2004, 2005, Stewart et al. 2014) were able to delineate geographically or host-restricted lineages of Alternaria, indicating the potential for phylogeographic studies. In contrast to plant pathogenic fungi, or fungi that have restricted geographic and host ranges, airborne fungi have been neglected as subjects for phylogeographic and population genetic studies (Slippers et al. 2005). It is generally believed that such fungi would display a lack of population subdivision due to their ease of spread, and that diversity levels would be extremely high due to high migration rates. Thus, the lack of data on the population genetics of non-pathogenic airborne fungi can be ascribed to these untested assumptions.

Few studies have investigated indoor Alternaria isolates specifically, although multiple studies mention the detection of Alternaria in the indoor environment (Solomon 1975, Li \& Kendrick 1995, de Ana et al. 2006). One large study of dust-borne A. alternata allergens in USA homes assessed the concentration of Alternaria allergens in dust with a polyclonal anti-alternaria antibody assay (Salo et al. 2005). That study revealed that exposure to $A$. alternata allergens is common, and that residential characteristics such as smoking, mold and moisture problems, and cleaning frequencies influence the indoor antigen levels in house dust. Nonetheless, no reports exist on the genotypic or allelic composition of indoor Alternaria
isolates from the USA. In addition to the few studies on indoor Alternaria species, more studies were performed on Aspergillus and Penicillium species. These two genera are poorly represented in outdoor air, but they are frequently isolated indoors (Scott et al. 2004, 2007, Araujo et al. 2010, Henk et al. 2011). A study on the genotypic variation in ca. 200 Penicillium chrysogenum strains from Canadian homes showed no evidence of recombination, indicating a strictly clonal population (Scott et al. 2004). Additionally, a study on the genotypic variation of the Penicillium brevicompactum group in house dust in Canada revealed that the two predominant taxa, P. brevicompactum and P. bialowiezense, also showed a predominantly clonal mode of reproduction (Scott et al. 2007).

Sexual reproduction in filamentous fungi is controlled by the mating-type (or MAT) locus (Coppin et al. 1997, Turgeon 1998). These mating-type loci have been identified from several asexual fungi based on PCR and whole genome sequencing (e.g. Sharon et al. 1996, Pöggeler 2002, Goodwin et al. 2003, Paoletti et al. 2005, Groenewald et al. 2006, Woo et al. 2006). The discovery of cryptic sexual cycles is important in understanding the evolution of fungal diversity. Alternaria is considered to be an asexual fungal genus; however, the connection to a sexual morph, formerly called Lewia, is known for some species (Simmons 1986, 2007). With the recent division of the genus into sections, these sexual connections seem to be restricted to specific sections (Lawrence et al. 2013, Chapter 2). However, the mating-type loci have also been identified from several Alternaria spp. which are supposedly asexual (Arie et al. 2000, Berbee et al. 2003, Linde et al. 2010, Stewart et al. 2011).

The first aim of the current study was to identify which Alternaria species are present in the indoor environment in the USA, by sequencing two protein-coding genes and one nontranslated locus. Secondly, we wanted to investigate the molecular diversity of indoor Alternaria isolates in the USA, by genotyping and population genetic inference of the section Alternaria isolates, using five microsatellite markers (Tran-Dinh \& Hocking 2006). A third aim was to investigate whether alleles at these five microsatellite loci are randomly associated, i.e. to test for recombination.

## MATERIALS AND METHODS

## Isolates and DNA extraction

Isolates were collected throughout the USA over a period of 6 months from December 2011 to May 2012 (Table 1). Most of the samples ( $137 / 156$ ) were collected as malt extract agar (MEA) settle plates by homeowners from their own homes. The MEA plates were purchased by homeowners from hardware stores and sent to EMSL Analytical, Inc. for identification after exposure to indoor air. Ten air samples were collected with a single stage bio-aerosol impaction sampler (EMSLVP-400 Microbial Sampler), three were swab samples and four were dust samples (Table 1). The media used for fungal isolation was MEA. No further information is available on the individual homes. For the microsatellite typing experiment, 37 reference isolates were included (Table 1). For DNA isolation, the isolates were grown on potato-carrot agar (Crous et al. 2009c) for 7d at ambient temperature ( $\sim 22^{\circ} \mathrm{C}$ ). Total genomic DNA was extracted using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions.

## PCR, sequencing and sequence analyses

The internal transcribed spacers (ITS) of the ribosomal DNA operon, including the 5.8 S rDNA gene, and a section of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene region were amplified from genomic DNA as described in Chapter 2 with the primers V9G (De Hoog \& Gerrits van den Ende 1998) and ITS4 (White et al. 1990) for the ITS region, and gpd1 and gpd2 (Berbee et al. 1999) for the GAPDH region. A section of the endopolygalacturonase (endoPG) gene was amplified with the primers PG3 and PG2b (Andrew et al. 2009). The PCR mixture consisted of $1 \mu \mathrm{l}$ genomic DNA ( ca. 50 ng ), $1 \times$ PCR reaction buffer (Bioline, Luckenwalde, Germany), $2 \mathrm{mM} \mathrm{MgCl} 2,20 \mu \mathrm{M}$ of each dNTP, $0.2 \mu \mathrm{M}$ of each primer, and 0.5 U Taq DNA polymerase (Bioline). The PCR program consisted of an initial denaturation step of 5 min at $94^{\circ} \mathrm{C}$ followed by 40 cycles of 30 s at $94^{\circ} \mathrm{C}, 30 \mathrm{~s}$ at $50^{\circ} \mathrm{C}$ and 30 s at $72^{\circ} \mathrm{C}$ and a final elongation step of 7 min at $72^{\circ} \mathrm{C}$. The PCR reactions were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California), in a total volume of $12.5 \mu$ l. PCR amplicons were sequenced in both directions using the PCR primers and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, and analysed using an ABI Prism 3730xl DNA Analyzer (Applied Biosystems). Consensus sequences were assembled from forward and reverse sequences using the BioNumerics v. 4.61 software package (Applied Maths, St-Martens-Latem, Belgium).

Sequence alignments were generated with MAFFTv. 7 (Katoh\& Standley 2013), and manually adjusted where necessary. A Bayesian inference analysis was conducted with MrBayes v. 3.2.1 (Huelsenbeck \& Ronquist 2001, Ronquist \& Huelsenbeck 2003) on the individual datasets. The K80 model with gamma distribution was used for the ITS region, and the GTR-model with gamma distribution for the GAPDH and endoPG regions, as suggested by the on-line tool FindModel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). The two Markov Chain Monte Carlo (MCMC) analyses used four chains and started from a random tree topology. The analysis ran with the sample frequency set at 1000 and the temperature value of the heated chain at 0.05 and stopped when the average standard deviation of split frequencies fell below 0.01 . Burn-in was set to $25 \%$, after which the likelihood values were stationary. The convergence of chains was verified with Tracer v. 1.5.0 (Rambaut \& Drummond 2009), and TreeView v. 1.6.6 (Page 1996) was used to visualise the phylogenetic tree. Both the sequence alignments and phylogenetic trees were deposited in TreeBASE (http://www.treebase.org).

## Microsatellite typing

Five primer pairs previously designed for A. alternata (Tran-Dinh \& Hocking 2006; Table 2) were used to characterize the indoor Alternaria section Alternaria population from the USA, together with 37 reference isolates (Table 1). By performing a genomic search of the primer sequences against a draft $A$. alternata genome (Chapter 5), the relative positions of the microsatellites on the genome were located. From each primer pair, one primer was labelled with the Fluorobrite oligo FAM (loci AEM3 and AEM5), SOL (locus AEM6) or ZEL (loci AEM9 and AEM13) (Biolegio BV, Nijmegen, The Netherlands; Table 2). Loci AEM3 / AEM5 and AEM9 / AEM13 were amplified in a multiplex PCR. The PCR mixture consisted of $1 \mu \mathrm{DNA}$ ( $c a .50 \mathrm{ng}$ ), $1 \times$ PCR buffer (Bioline), $40 \mu \mathrm{M}$ of each dNTP, $1.6 \mathrm{mM} \mathrm{MgCl}_{2}, 0.2 \mu \mathrm{M}$ of each primer, and 0.25 U Taq polymerase (Bioline) in a total volume of $12.5 \mu$. The amplification was performed on a 2720 Thermal Cycler (Applied Biosystems) and consisted of a 5 min initial denaturation step $\left(94^{\circ} \mathrm{C}\right)$ followed by 35 cycles of 30 s at $94^{\circ} \mathrm{C}, 55^{\circ} \mathrm{C}$ and $72^{\circ} \mathrm{C}$, and a final 7 min elongation
Table 1. Isolates used in this study with the substrate, locality and date they were collected, and their sequence type (ST) and eBURST group based on microsatellite data.

| Isolate number ${ }^{1}$ | Substrate | Locality | Date | Name | $\mathbf{S T}^{3}$ | eBURST group ${ }^{3}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CPC 22417 | Air ${ }^{2}$, bedroom 2nd floor | USA, NJ | Dec. 2012 | A. arborescens SC | 12 | singleton |
| CPC 22418 | Dust, carpet | USA, PA | Dec. 2012 | A. alternata | 39 | singleton |
| CPC 22419 | Air, bedroom | USA, CA | Jan. 2013 | A. alternata | 79 | singleton |
| CPC 22420 | Air, bedroom | USA, MD | Jan. 2013 | A. alternata | 44 | 1 |
| CPC 22421 | Air, bathroom | USA, TX | Jan. 2013 | A. alternata | 18 | 1 |
| CPC 22422 | Air, recreational vehicle | USA, CA | Jan. 2013 | sect. Infectoriae | $n \boldsymbol{a}$ | $n \boldsymbol{a}$ |
| CPC 22423 | Air, bedroom | USA, NJ | Dec. 2012 | A. arborescens SC | 106 | singleton |
| CPC 22424 | Air, living room | USA, KS | Dec. 2012 | A. alternata | 167 | singleton |
| CPC 22425 | Air, office | USA, NJ | Dec. 2012 | A. alternata | 118 | 19 |
| CPC 22426 | Air, office | USA, MI | Dec. 2012 | A. alternata | 44 | 1 |
| CPC 22427 | Air, kitchen | USA, MD | Dec. 2012 | A. alternata | 158 | singleton |
| CPC 22428 | Air, bedroom | USA, WI | Dec. 2012 | A. alternata | 86 | 3 |
| CPC 22429 | Air, living room | USA, NJ | Jan. 2013 | A. alternata | 134 | 2 |
| CPC 22430 | Air, class room | USA, TX | Jan. 2013 | A. alternata | 105 | 1 |
| CPC 22431 | Air, office | USA, CO | Dec. 2012 | A. alternata | 91 | 1 |
| CPC 22432 | Air, bedroom | USA, IL | Dec. 2012 | A. alternata | 59 | 1 |
| CPC 22433 | Air, basement | USA, NJ | Dec. 2012 | A. alternata | 52 | 1 |
| CPC 22434 | Air, 2nd floor | USA, NJ | Jan. 2013 | A. alternata | 133 | 2 |
| CPC 22435 | Air ${ }^{2}$, office | USA, NY | Jan. 2013 | A. alternata | 61 | 1 |
| CPC 22436 | Air, bathroom | USA, MD | Jan. 2013 | A. alternata | 47 | 1 |
| CPC 22437 | Swab, store | USA, NY | Jan. 2013 | A. alternata | 157 | singleton |
| CPC 22438 | Air, basement | USA, CT | Jan. 2013 | A. alternata | 145 | singleton |
| CPC 22439 | Air, living room | USA, NJ | Jan. 2013 | A. alternata | 111 | 1 |
| CPC 22440 | Air, bedroom | USA, WA | Jan. 2013 | sect. Pseudoulocladium | $n \boldsymbol{a}$ | $n \boldsymbol{a}$ |
| CPC 22441 | Dust, rug | USA, PA | Jan. 2013 | A. alternata | 82 | singleton |

Table 1. (Continued)

| Isolate number ${ }^{1}$ | Substrate | Locality | Date | Name | ST ${ }^{3}$ | eBURST group ${ }^{3}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CPC 22970 | Dust, rug | USA, PA | Jan. 2013 | A. alternata | 67 | singleton |
| CPC 22971 | Air, class room | USA, NY | Jan. 2013 | A. alternata | 68 | 1 |
| CPC 22972 | Air, hallway | USA, CA | Jan. 2013 | A. alternata | 71 | 1 |
| CPC 22973 | Air, hallway | USA, CA | Jan. 2013 | A. alternata | 23 | 10 |
| CPC 22974 | Air, kitchen | USA, GA | Jan. 2013 | A. alternata | 141 | 15 |
| CPC 22975 | Air, kitchen | USA, SD | Jan. 2013 | A. alternata | 32 | singleton |
| CPC 22976 | Air, basement | USA, OH | Jan. 2013 | A. alternata | 49 | singleton |
| CPC 22977 | Air, living room | USA, PA | Jan. 2013 | A. alternata | 46 | 1 |
| CPC 22978 | Air, living room | USA, MD | Jan. 2013 | A. arborescens SC | 98 | singleton |
| CPC 22979 | Air, bedroom | USA, OK | Jan. 2013 | A. alternata | 174 | 1 |
| CPC 22980 | Air, living room | USA, CA | Jan. 2013 | A. alternata | 74 | singleton |
| CPC 22981 | Air, living room | USA, OH | Jan. 2013 | A. alternata | 159 | singleton |
| CPC 22982 | Air, living room | USA, CA | Jan. 2013 | A. alternata | 62 | 1 |
| CPC 22983 | Air, bedroom | USA, IL | Jan. 2013 | A. alternata | 62 | 1 |
| CPC 22984 | Air, basement | USA, NY | Jan. 2013 | A. alternata | 80 | singleton |
| CPC 22985 | Air, office | USA, AZ | Jan. 2013 | A. alternata | 125 | 1 |
| CPC 22986 | Swab, bedroom | USA, TX | Jan. 2013 | A. alternata | 166 | 1 |
| CPC 22987 | $\mathrm{Air}^{2}$, outside | USA, DE | Feb. 2013 | A. alternata | 89 | 1 |
| CPC 22988 | $\mathrm{Air}^{2}$, office | USA, DE | Feb. 2013 | A. alternata | 85 | 3 |
| CPC 22989 | Air ${ }^{2}$, warehouse | USA, DE | Feb. 2013 | A. alternata | 41 | 9 |
| CPC 22990 | $\mathrm{Air}^{2}$, office | USA, AZ | Feb. 2013 | A. alternata | 127 | singleton |
| CPC 22991 | $\mathrm{Air}^{2}$, elevator | USA, MO | Feb. 2013 | A. alternata | 171 | singleton |
| CPC 22992 | Air, living room | USA, IL | Feb. 2013 | A. alternata | 64 | 7 |
| CPC 22993 | Dust, carpet | USA, MD | Feb. 2013 | A. arborescens SC | 146 | 14 |
| CPC 22994 | Air, office | USA, GA | Feb. 2013 | A. alternata | 128 | singleton |
| CPC 22995 | Air, office | USA, TX | Feb. 2013 | A. alternata | 125 | 1 |
| CPC 22996 | Air, garage | USA, TX | Feb. 2013 | A. alternata | 153 | singleton |

Table 1. (Continued)

| Isolate number ${ }^{1}$ | Substrate | Locality | Date | Name | $\mathbf{S T}^{3}$ | eBURST group ${ }^{3}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CPC 22997 | Air, bedroom | USA, CO | Feb. 2013 | A. alternata | 115 | singleton |
| CPC 22998 | Air, bedroom | USA, TX | Feb. 2013 | A. arborescens SC | 172 | 12 |
| CPC 22999 | Air, bedroom | USA, FL | Feb. 2013 | A. alternata | 47 | 1 |
| CPC 23000 | Air, living room | USA, WA | Feb. 2013 | A. arborescens SC | 104 | singleton |
| CPC 23001 | Air, bedroom | USA, OR | Feb. 2013 | A. alternata | 19 | 1 |
| CPC 23002 | Air, bedroom | USA, IL | Feb. 2013 | A. alternata | 90 | 1 |
| CPC 23003 | Air, outside | USA, OK | Feb. 2013 | A. alternata | 101 | singleton |
| CPC 23004 | Air, living room | USA, TX | Feb. 2013 | A. alternata | 138 | 1 |
| CPC 23005 | Air, bedroom | USA, IL | Feb. 2013 | A. alternata | 148 | singleton |
| CPC 23006 | Air, bedroom | USA, PA | Feb. 2013 | A. alternata | 122 | 17 |
| CPC 23007 | Air, bedroom | USA, CA | Feb. 2013 | A. alternata | 100 | singleton |
| CPC 23008 | Air, bathroom | USA, TX | Feb. 2013 | A. alternata | 161 | singleton |
| CPC 23009 | Air, storage room | USA, MD | Feb. 2013 | A. alternata | 16 | singleton |
| CPC 23010 | Air, kitchen | USA, TX | Feb. 2013 | A. arborescens SC | 151 | 14 |
| CPC 23011 | Air, bedroom | USA, MO | Feb. 2013 | A. alternata | 50 | 1 |
| CPC 23012 | Air, bedroom | USA, FL | Feb. 2013 | A. alternata | 58 | 1 |
| CPC 23013 | Air, bedroom | USA, FL | Feb. 2013 | A. alternata | 47 | 1 |
| CPC 23014 | Air, living room | USA, GA | Feb. 2013 | A. alternata | 96 | 1 |
| CPC 23015 | Air, dining room | USA, NJ | Feb. 2013 | A. arborescens SC | 112 | singleton |
| CPC 23016 | Air, bedroom | USA, GA | Feb. 2013 | A. alternata | 117 | 19 |
| CPC 23017 | Air, office | USA, MS | Feb. 2013 | A. alternata | 123 | 17 |
| CPC 23018 | Air, bathroom | USA, TX | Feb. 2013 | A. alternata | 124 | 1 |
| CPC 23019 | Air, living room | USA, ME | Feb. 2013 | A. alternata | 71 | 1 |
| CPC 23020 | Air, bedroom | USA, IL | Feb. 2013 | A. alternata | 69 | 1 |
| CPC 23021 | Air, bathroom | USA, PA | Feb. 2013 | A. alternata | 135 | singleton |
| CPC 23022 | Air, office | USA, CA | Feb. 2013 | A. alternata | 26 | singleton |
| CPC 23023 | Air, bedroom | USA, TX | Mar. 2013 | A. alternata | 27 | 1 |

Table 1. (Continued).

| Isolate number ${ }^{1}$ | Substrate | Locality | Date | Name | $\mathbf{S T}^{3}$ | eBURST group ${ }^{3}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CPC 23024 | Air, bathroom | USA, CA | Mar. 2013 | A. arborescens SC | 15 | 12 |
| CPC 23025 | Air, bathroom | USA, MA | Mar. 2013 | A. alternata | 59 | 1 |
| CPC 23026 | Air, bedroom | USA, CO | Mar. 2013 | A. arborescens SC | 8 | 16 |
| CPC 23027 | Air, hallway | USA, MI | Mar. 2013 | A. alternata | 126 | 1 |
| CPC 23028 | Air, living room | USA, TX | Mar. 2013 | A. alternata | 136 | singleton |
| CPC 23029 | Air, bedroom | USA, IN | Mar. 2013 | A. alternata | 114 | singleton |
| CPC 23030 | Air, bedroom | USA, LA | Mar. 2013 | A. alternata | 162 | 8 |
| CPC 23031 | Air, bedroom | USA, NJ | Mar. 2013 | A. alternata | 170 | 13 |
| CPC 23032 | Air, break room | USA, TX | Mar. 2013 | A. alternata | 88 | 1 |
| CPC 23033 | Air, family room | USA, GA | Mar. 2013 | A. alternata | 20 | 1 |
| CPC 23034 | Air, bedroom | USA, CA | Mar. 2013 | A. alternata | 65 | 7 |
| CPC 23035 | Air, bathroom | USA, TX | Mar. 2013 | A. alternata | 129 | 1 |
| CPC 23036 | Air, bedroom | USA, PA | Mar. 2013 | A. alternata | 140 | 1 |
| CPC 23037 | $\mathrm{Air}^{2}$, office | USA, AZ | Mar. 2013 | A. alternata | 40 | 9 |
| CPC 23038 | Air, garage | USA, NJ | Mar. 2013 | A. alternata | 48 | 1 |
| CPC 23039 | Air, kitchen | USA, TX | Mar. 2013 | A. alternata | 95 | singleton |
| CPC 23040 | Air, outside | USA, CA | Mar. 2013 | A. arborescens SC | 11 | 18 |
| CPC 23041 | Air, bathroom | USA, TX | Mar. 2013 | A. alternata | 150 | 2 |
| CPC 23042 | Air, living room | USA, PA | Mar. 2013 | A. alternata | 56 | 1 |
| CPC 23043 | Air, living room | USA, CA | Mar. 2013 | A. arborescens SC | 9 | singleton |
| CPC 23044 | Air, office | USA, NE | Mar. 2013 | A. alternata | 45 | 1 |
| CPC 23045 | Air, living room | USA, GA | Mar. 2013 | A. alternata | 142 | 15 |
| CPC 23046 | Air, office | USA, IL | Mar. 2013 | A. alternata | 139 | 1 |
| CPC 23047 | Air, bedroom | USA, KY | Mar. 2013 | A. alternata | 144 | singleton |
| CPC 23048 | Air, bathroom | USA, SC | Mar. 2013 | A. alternata | 87 | 3 |
| CPC 23049 | Air, dining room | USA, NM | Mar. 2013 | A. alternata | 77 | singleton |
| CPC 23050 | Air, class room | USA, MO | Mar. 2013 | A. alternata | 78 | 1 |

Table 1. (Continued).

| Isolate number ${ }^{1}$ | Substrate | Locality | Date | Name | ST ${ }^{3}$ | eBURST group ${ }^{3}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CPC 23051 | Air, bedroom | USA, TX | Mar. 2013 | A. alternata | 107 | 1 |
| CPC 23052 | Air, office | USA, FL | Mar. 2013 | A. alternata | 149 | 2 |
| CPC 23053 | Air ${ }^{2}$, bathroom | USA, NJ | Mar. 2013 | sect. Pseudoulocladium | na | $n \boldsymbol{a}$ |
| CPC 23054 | Air, outside | USA, MD | Mar. 2013 | A. alternata | 160 | singleton |
| CPC 23055 | Air, bedroom | USA, TN | Apr. 2013 | A. alternata | 113 | 13 |
| CPC 23056 | Air, living room | USA, CT | Apr. 2013 | A. alternata | 70 | 1 |
| CPC 23057 | Air, outside | USA, CA | Apr. 2013 | A. arborescens SC | 7 | 16 |
| CPC 23058 | Air, bedroom | USA, AL | Apr. 2013 | A. alternata | 53 | 1 |
| CPC 23059 | Air, outside | USA, GA | Apr. 2013 | A. alternata | 132 | 2 |
| CPC 23060 | Air, bathroom closet | USA, NJ | Apr. 2013 | A. alternata | 34 | 4 |
| CPC 23061 | Air, bedroom | USA, TX | Apr. 2013 | A. alternata | 169 | singleton |
| CPC 23062 | Air, bedroom | USA, TX | Apr. 2013 | A. alternata | 147 | singleton |
| CPC 23063 | Air, bedroom | USA, FL | Apr. 2013 | A. burnsii | 5 | 5 |
| CPC 23064 | Air, office | USA, FL | Apr. 2013 | A. alternata | 60 | 1 |
| CPC 23065 | Air, bedroom | USA, NY | Apr. 2013 | A. alternata | 109 | singleton |
| CPC 23066 | Air, bedroom | USA, CA | Apr. 2013 | A. alternata | 81 | singleton |
| CPC 23067 | Air, class room | USA, NC | Apr. 2013 | A. alternata | 31 | singleton |
| CPC 23068 | Air, living room | USA, NJ | Apr. 2013 | A. alternata | 37 | 4 |
| CPC 23069 | Air, family room | USA, CA | Apr. 2013 | A. alternata | 75 | 11 |
| CPC 23070 | Air, utility room | USA, MS | Apr. 2013 | A. alternata | 55 | 1 |
| CPC 23071 | Air, living room | USA, CA | Apr. 2013 | A. alternata | 102 | 6 |
| CPC 23072 | Air, basement | USA, PA | Apr. 2013 | A. alternata | 49 | singleton |
| CPC 23073 | Air, living room | USA, MO | Apr. 2013 | A. alternata | 72 | 1 |
| CPC 23074 | Air, bedroom | USA, MO | Apr. 2013 | A. alternata | 168 | 1 |
| CPC 23075 | Air, dining room | USA, TX | Apr. 2013 | A. alternata | 102 | 6 |
| CPC 23076 | Air, bedroom | USA, FL | Apr. 2013 | A. alternata | 84 | 3 |
| CPC 23077 | Air, kitchen | USA, IL | Apr. 2013 | A. alternata | 116 | 1 |

Table 1. (Continued).

| Isolate number ${ }^{1}$ | Substrate | Locality | Date | Name | $\mathbf{S T}^{3}$ | eBURST group ${ }^{3}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CPC 23078 | Air, living room | USA, NY | Apr. 2013 | A. alternata | 137 | singleton |
| CPC 23079 | Air, bathroom | USA, FL | Apr. 2013 | A. alternata | 43 | 1 |
| CPC 23080 | Air, bedroom | USA, MI | Apr. 2013 | A. alternata | 173 | singleton |
| CPC 23081 | Air, bedroom | USA, AZ | Apr. 2013 | A. alternata | 76 | 11 |
| CPC 23082 | Swab, wine barrel | USA, PA | Apr. 2013 | A. alternata | 22 | 1 |
| CPC 23083 | Air, living room | USA, GA | May 2013 | A. alternata | 30 | 2 |
| CPC 23084 | Air, bathroom | USA, IL | May 2013 | A. alternata | 163 | singleton |
| CPC 23085 | Air $^{2}$, warehouse | USA, DE | May 2013 | A. alternata | 41 | 9 |
| CPC 23086 | Air, bathroom | USA, CA | May 2013 | A. alternata | 93 | singleton |
| CPC 23087 | Air, basement | USA, PA | May 2013 | A. alternata | 36 | 4 |
| CPC 23088 | Air, bedroom | USA, FL | May 2013 | A. alternata | 143 | singleton |
| CPC 23089 | Air, bedroom | USA, CA | May 2013 | A. arborescens SC | 10 | 18 |
| CPC 23090 | Air, kitchen | USA, AZ | May 2013 | A. alternata | 165 | singleton |
| CPC 23091 | Air, bedroom | USA, IA | May 2013 | A. alternata | 35 | 4 |
| CPC 23092 | Air, bedroom | USA, RI | May 2013 | A. alternata | 164 | singleton |
| CPC 23093 | Air, living room | USA, GA | May 2013 | A. alternata | 73 | singleton |
| CPC 23094 | Air, bathroom | USA, CA | May 2013 | A. alternata | 25 | 10 |
| CPC 23095 | Air, bathroom | USA, VA | May 2013 | A. arborescens SC | 152 | singleton |
| CPC 23096 | Air, office | USA, TX | May 2013 | A. alternata | 130 | 1 |
| CPC 23097 | Air, office | USA, OK | May 2013 | A. alternata | 155 | singleton |
| CPC 23098 | Air, basement | USA, MA | May 2013 | A. alternata | 131 | 2 |
| CPC 23099 | Leaf, green house | USA, NC | May 2013 | A. alternata | 102 | 6 |
| CPC 23100 | Leaf, green house | USA, NC | May 2013 | A. alternata | 110 | 6 |
| CBS 101.13 | Unknown | Unknown | < Jan. 1913 | A. arborescens SC | 8 | 16 |
| CBS 103.33 | Soil | Egypt | < Jan. 1933 | A. alternata | 24 | 10 |
| CBS 107.38 | Cuminum cyminum | Unknown | < Dec. 1938 | A. burnsii | 3 | 5 |
| CBS 117.44 | Godetia sp. | Denmark | Jul. 1942 | A. alternata | 28 | singleton |

Table 1. (Continued)

| Isolate number ${ }^{1}$ | Substrate | Locality | Date | Name | ST ${ }^{3}$ | eBURST group ${ }^{3}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CBS 194.86 | Quercus sp. | USA | 1953 | A. alternata | 33 | singleton |
| CBS 195.86 | Euphorbia esula | Canada | 1982 | A. alternata | 103 | 6 |
| CBS 479.90 | Citrus unshiu | Japan | 1968 | A. alternata | 42 | 9 |
| CBS 632.93 | Pyrus pyrifolia | Japan | Jul. 1990 | A. gaisen | 14 | 8 |
| CBS 540.94 | Nicotiana tabacum | USA | < Nov. 1971 | A. longipes | 97 | singleton |
| CBS 916.96 | Arachis hypogaea | India | Dec. 1980 | A. alternata | 99 | singleton |
| CBS 918.96 | Dianthus chinensis | UK | Feb. 1981 | A. alternata | 21 | 1 |
| CBS 102595 | Citrus jambhiri | USA | < Jul. 1997 | A. alternata | 54 | 1 |
| CBS 102596 | Citrus jambhiri | USA | < Jul. 1997 | A. alternata | 92 | 1 |
| CBS 102597 | Minneola tangelo | USA | < Aug. 1997 | A. gossypina | 17 | singleton |
| CBS 102598 | Minneola tangelo | USA | < Feb. 1998 | A. alternata | 175 | 1 |
| CBS 102599 | Minneola tangelo | Turkey | May 1996 | A. alternata | 51 | 1 |
| CBS 102600 | Citrus reticulata | USA | Jun. 1975 | A. alternata | 57 | 1 |
| CBS 102601 | Minneola tangelo | Colombia | < Nov. 1996 | A. gossypina | 17 | singleton |
| CBS 102602 | Minneola tangelo | Turkey | May 1996 | A. alternata | 51 | 1 |
| CBS 102604 | Minneola tangelo | Israel | < Nov. 1996 | A. alternata | 66 | singleton |
| CBS 102605 | Solanum lycopersicum | USA | Apr. 1990 | A. arborescens SC | 8 | 16 |
| CBS 118404 | Iris sp. | New Zealand | Jan. 2001 | A. iridiaustralis | 6 | singleton |
| CBS 118488 | Pyrus pyrifolia | Japan | Jul. 1990 | A. gaisen | 13 | 8 |
| CBS 118809 | Alstroemeria sp. | Australia | Jul. 2005 | A. alstroemeriae | 119 | singleton |
| CBS 118810 | Beta vulgaris var. cicla | Kenya | 2001 | A. betae-kenyensis | 1 | singleton |
| CBS 118811 | Brassica oleracea | USA | Apr. 1982 | A. alternata | 94 | singleton |
| CBS 118812 | Daucus carota | USA | Jan. 1984 | A. alternata | 83 | 3 |
| CBS 118814 | Solanum lycopersicum | USA | Jun. 1996 | A. alternata | 156 | 3 |
| CBS 118816 | Rhizophora mucronata | India | Oct. 1995 | A. burnsii | 2 | 5 |
| CBS 118817 | Tinospora cordifolia | India | Sep. 1987 | A. burnsii | 4 | 5 |

Table 1. (Continued)

| Isolate number ${ }^{1}$ | Substrate | Locality | Date | Name | $\mathbf{S T}^{3}$ | eBURST group ${ }^{3}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| CBS 118818 | Vaccinium sp. | USA | Oct. 1973 | A. alternata | 108 | 1 |
| CBS 119399 | Minneola tangelo | USA | Dec. 1980 | A. alternata | 121 | singleton |
| CBS 119408 | Euphorbia esula | USA | Nov. 1992 | A. alternata | 38 | 4 |
| CBS 119543 | Citrus paradisi | USA | Jun. 1947 | A. alternata | 63 | 7 |
| CBS 121454 | Cuscuta sp. | USA | Aug. 1997 | A. alternata | 154 | singleton |
| CBS 121455 | Broussonetia papyrifera | China | Sep. 1996 | A. alternata | 29 | 2 |
| CBS 124392 | Solanum melongena | China | Unknown | A. alternantherae | 120 | singleton |
| ${ }^{1}$ CBS: Culture collection of the Centraalbureau voor Schimme Crous, Utrecht, The Netherlands. <br> ${ }^{2}$ Collected with a single stage bio-aerosol impaction sampler. ${ }^{3}$ na: not analysed. |  |  |  |  |  |  |
| Table 2. PCR primer sequences, repeat motifs, number of alleles and allele distribution observed for microsatellite markers in Alternaria Alternaria based on 153 isolates. |  |  |  |  |  |  |
| Locus | Primer sequence ( $\left.5^{\prime}-3^{\prime}\right)^{1}$ |  | repeat motif No. |  | No. of alleles | Allele distribution |
| AEM3 | F: TGA TCC CAC GTC ACA GAA AG <br> R: FGGT TGT CCA AGT ACC CCA TAG A |  | (AAG) ${ }_{9}$ |  |  | Uneven |
| AEM5 | F: ${ }^{\text {FTAC AGA CGG AGG }}$ <br> R: CAC AGC TCG TCA T | $\begin{aligned} & \mathrm{C} \mathrm{AC} \\ & \mathrm{G} \text { TA } \end{aligned}$ | $(\mathrm{GAA})_{10}$ |  |  | Even |
| AEM6 | F: TGA CGA GCT GTG A <br> R: ${ }^{\text {s }}$ CGT GTG TAG GGT | $\begin{aligned} & \text { Г GT } \\ & \text { T CTC } \end{aligned}$ | $(\mathrm{CA})_{5}(\mathrm{CT}$ |  |  | Uneven |
| AEM9 | F: GAA GCC CAT TCC A R: ${ }^{\text {Z GCT CCA TCT CCC }}$ | A ACA | $(\mathrm{CAA})_{12}$ |  |  | Uneven |
| AEM13 | F: TGC GAA ACC GTG <br> R: ${ }^{\text {TCG }}$ GAA ATG GCT |  | $(\mathrm{GAC})_{7}(\mathrm{GAA})_{38}$ |  |  | Even |

${ }^{1 \mathrm{~F}, \mathrm{~S}}{ }^{\text {or }}{ }^{\mathrm{z}}$ indicates the use of respectively FAM, SOL or ZEL as fluorescent lable.
step $\left(72^{\circ} \mathrm{C}\right)$. For fragment analysis, the PCR products were diluted 1:1000 and combined per biological sample, which resulted in one well per isolate for the fragment analysis. MCLAB's Orange Size Standard (Nimagen, Nijmegen, The Netherlands) was used as internal marker. Samples were electrophoresed using an ABI Prism 3730x1 DNA Analyzer (Applied Biosystems), and analysed with the freeware Peak Scanner v. 1.0 (Applied Biosystems). Individual alleles at each locus were assigned using fragment lengths.

## Population genetic analyses

The online program eBURST v. 3 (http://eburst.mlst.net/v3; Feil et al. 2004) was used to identify clusters of closely related genotypes. The allelic profiles were assigned to sequence types (STs; Table 1), and eBURST identified groups of STs that only differed at one locus (known as single locus variants).

For subsequent population genetic analyses, the A. alternata isolates were divided into four artificial subpopulations representing four quadrants of the USA. Isolates from NE-USA were excluded due to small sample size. The program MultiLocus v. 1.3 (Agapow \& Burt 2001) was used to simulate genotypic diversity against the number of loci ( 1000 randomizations per locus combination), in order to test whether sampling was sufficient for population genetic analyses. The same software was used to calculate the genotypic diversity and linkage disequilibrium in subpopulations of isolates ( 10000 data randomizations). For this analysis, locus AEM6 was excluded due to the fact that it was physically linked to locus AEM5 and was also less polymorphic than that locus. The Index of Association $\left(I_{A}\right)$ between alleles at different loci was normalized as $\bar{r}_{d}$ as an indication of random association between loci. The null hypothesis for this test was that alleles are randomly associated, and deviation from random association is measured as a confidence interval. The $\theta$-values of population differentiation between pairwise combinations of subpopulations, i.e. SW-USA, NE-USA, and SE-USA (Fig. 1), were used to estimate the pairwise number of migrants per generation (Slatkin 1995), which equates to gene flow ( $\hat{M}$ ).

The Stoddart \& Taylor (1988) genotypic diversity was manually calculated for isolates from each included quadrant of the USA, and the genotypic diversity of each subpopulation was normalized with sample size to yield $\hat{G}$ (the percentage of maximum genotypic diversity), which can be used to make inter-sample comparisons. The significance of differences between $\hat{G}$ values was assessed using a two-tailed $t$-test at a significance level of $99 \%(P=0.01)$ with $N_{1}$ $+N_{2}-2$ degrees of freedom, where $N$ is the sample size.

In order to assess diversity that is independent of genotypes, the allelic (gene) diversity (Nei 1973) was calculated. This statistic provides an indication of heterozygosity, or the probability of obtaining two different alleles at a locus when two individuals are randomly sampled from a haploid population. $\mathrm{H} \rightarrow 1$ for diverse populations, while $\mathrm{H} \rightarrow 0$ for populations that display allelic homogeneity. Additionally, the level of uniqueness ( $\varphi$ ) (Van der Merwe et al. 2012) for each subpopulation was calculated. This statistic estimates the probability of sampling a unique (private) allele belonging to a subpopulation, when a random individual is drawn from the total population. In other words, $\varphi$ is an indication of allelic segregation in a subpopulation.


Fig. 1. Map of the mainland USA (Mercator projection) indicating the four artificially defined quadrants using different colors, and the general north-easterly direction of the antitrade winds over the subcontinent (grey arrows). Numbers in black filled circles are the numbers of isolates from each state. The boxed insert depicts gene flow estimations between the south-west, south-east and north-east quadrants. Diameters of the circles are proportionate to the level of uniqueness $(\varphi)$ of each of the subpopulations. The north-west quadrant was excluded from these analyses due to lack of a sufficient number of isolates.

## RESULTS

## Phylogeny

From the 193 included isolates we were not able to amplify the endoPG sequences from five isolates (CPC 22422, CPC 22440, CPC 23053, CPC 23063 and A. alternantherae CBS 124392). The sequences of the ITS ( 554 characters), GAPDH ( 580 characters) and endoPG ( 448 characters) gene regions consisted of respectively 58,88 and 47 unique site patterns. After discarding the burn-in, the Bayesian analysis resulted in respectively 4308, 5200 and 4218 trees from both runs. Based on their ITS, GAPDH and endoPG sequences, 153 of the 156 isolates (i.e. $98 \%$ ) belonged to section Alternaria, while two isolates belonged to section Pseudoulocladium (CPC 22440, CPC 23053), and one belonged to section Infectoriae (CPC 22422) (Table 1). From the 153 isolates that belonged to section Alternaria, CPC 23063 could be assigned to A. burnsii and 15 other isolates could be assigned to the $A$. arborescens species complex (AASC). The remaining 137 isolates were identified as $A$. alternata. Both the GAPDH and ITS phylogeny could distinguish the section Alternantherae, section Pseudoulocladium and section Infectoriae isolates from the section Alternaria isolates. The endoPG locus from the isolates outside section Alternaria could not be amplified. Within section Alternaria the ITS phylogeny could only distinguish $A$. betae-kenyensis, $A$. burnsii, $A$. iridiaustralis and $A$. longipes. The other five included Alternaria species, A. alstroemeriae, A. alternata, A. gaisen, A. gossypina, and
the AASC, all clustered together based on their ITS sequences. The GAPDH and endoPG phylogenies separated all included species in section Alternaria except AASC / A. alternata and A. gossypina / A. longipes, respectively. The clustering of the A. alternata isolates with respect to the other recognized species in section Alternaria was not consistent throughout the three sequenced genes, as inconsistent sub-clusters were formed.

## Microsatellite typing

Comparisons of microsatellite loci to a draft genome sequence revealed that three of the loci, namely AEM3, AEM9 and AEM13 each resided on a different genomic scaffold. Loci AEM5 and AEM6 resided on a single scaffold, and the AEM5-R and AEM6-F primers overlapped with 12 nt . We found 142 allelic profiles (or sequence types, ST) from the 153 collected isolates (Table 1). When the 37 reference isolates were included, 175 allelic profiles were observed. Loci AEM3 and AEM13 showed the largest number of alleles (Table 2). However, within AEM3 there was an uneven distribution of the different alleles, with allele 257 being observed in 58 of 153 isolates ( $\sim 35 \%$ ). For loci AEM6 and AEM9 the distribution across the different alleles was more unbalanced. At these loci, alleles 161 and 278 were observed in $\sim 70 \%$ of the isolates. For loci AEM5 and AEM13 there was an even distribution among the different alleles. The locus AEM13, which displayed the highest number of alleles and an even distribution of these alleles, contributed most to the genotypic variation, followed by AEM3 with a high number of alleles but with an uneven distribution. Loci AEM6 and AEM9 were the least informative loci, with a low number of alleles and an uneven distribution.

## Population genetic analyses

An eBURST analysis of 190 isolates ( 153 section Alternaria isolates and 37 reference isolates), representing 175 STs, resulted in 19 groups and 65 singletons (Fig. 2, Table 1). Group 1 was the largest, and included 62 isolates representing 54 STs (including eight reference isolates forming seven STs). Group 2 contained eight isolates representing eight STs (including 1 reference isolate) while group 3 contained six isolates and six STs (including 2 reference isolates). The remaining groups, namely $4-19$, included five or less isolates. The isolates assigned to the A. arborescens complex based on their endoPG sequence formed groups $12,14,16$, and 18 , while six isolates were singletons. The assignment of CPC 23063 to A. burnsii based on the GAPDH sequence is supported by the microsatellite data, since all $A$. burnsii isolates clustered in eBURST group 5. No correlation was found between the location and place of isolation and the eBURST groups assigned to the isolates based on their allelic profiles. Almost all eBURST groups contained isolates from different states in the USA and different places of isolation, e.g. bathroom, bedroom, kitchen. The only exceptions were group 13, which consists of two bedroom isolates, but isolated in two different states, and groups 15 and 18, which both consisted of two isolates from the same state, respectively Georgia and California, but from different places of isolation.

When the microsatellite alleles for the A. alternata (137) isolates were combined into multilocus genotypes (haplotypes), 126 distinct genotypes could be recovered. While most of these genotypes were observed only once, the most frequent genotype was observed three times. Modelling of the genotypic diversity $v s$. the number of loci revealed that both microsatellite loci and genotypes were adequately sampled to continue with population genetic analyses (Fig. 3). Index of Association values for three quadrants of the USA, namely the south-west,


Fig. 2. eBURST diagram of 190 Alternaria isolates. The numbers correspond to sequence type numbers, the size of the dot correlates to the number of isolates.

Genotypic diversity vs. Number of loci


Fig. 3. Results from modeling genotypic diversity against the number of loci. Each locus-combination was repeated 1000 times, resulting in a mean genotypic diversity for that combination. The graph reaches a plateau at four microsatellite loci, indicating that both the number of isolates and the number of loci were sufficient for population genetic analyses.


Fig. 4. Graph of linkage disequilibrium estimation densities ( $\bar{r}_{d}$ values, which are normalized Index of Association values) resulting from 10000 randomizations of each of the artificially-defined subpopulations, as well as for these three subpopulations combined. The observed linkage disequilibrium values are indicated using arrows, and these are inside the $95 \%$ confidence intervals of the distributions. Thus, the null hypothesis of random mating in these populations cannot be rejected.
north-east, and south-east quadrants (SW-USA, NE-USA, SE-USA) indicated that alleles were randomly associated for all three subpopulations, as well as for the metapopulation (Fig. 4, Table 3). Additionally, alleles of the two physically linked loci, namely AEM5 and AEM6, were in linkage disequilibrium ( $P<0.0001$ ), while all other loci were in pairwise equilibrium with each other and with AEM5. Population differentiation ( $\theta$; Table 4) was very low when pairwise combinations of these three subpopulations were analysed. Subsequently, the estimated numbers of migrants per generation $M$ were high between all three pair-wise combinations of subpopulations (Table 4). However, the migration rate between SE-USA and SW-USA ( $\hat{M}=$ 147,5 ) was much higher than the other two combinations, and the migration rate between SWUSA and NE-USA ( $\hat{M} \cong 32$ ) was the smallest.

The maximum likelihood estimator of genotypic diversity $(\hat{G})$ revealed that all subpopulations consisted of an extremely large diversity of genotypes (Table 3). No significant differences between the estimated $\hat{G}$-values could be detected using a two-tailed $t$-test. Gene diversity ( $\bar{H}$ ) values were $0.952,0.923$, and 0.916 for SW-USA, SE-USA, and NE-USA, respectively. Thus, alleles were most unevenly distributed in the SW-USA subpopulation ( $N=58$ ). An estimation of the level of uniqueness $(\varphi)$ of each subpopulation indicated that the SW-USA subpopulation was most unique ( $\varphi=0.915$ ), while the SE-USA and NE-USA subpopulations were equally unique ( $\varphi=0.564$ and $\varphi=0.568$, respectively).

Table 3. Summary statistics for indoor Alternaria alternata isolates from the USA.

| Statistic | All isolates ${ }^{\mathbf{1}}$ | South-West USA | North-East USA | South-East USA |
| :--- | :---: | :---: | :---: | :---: |
| Number of isolates, $N$ | 134 | 44 | 58 | 32 |
| Number of genotypes | 122 | 42 | 54 | 31 |
| ${ }^{2}$ Genotypic diversity, $\hat{G}$ | $81.71 \%$ | $91.67 \%$ | $87.88 \%$ | $94.12 \%$ |
| Number of alleles (all loci) | 104 | 72 | 66 | 52 |
| Gene diversity, $\bar{H}$ | 0.968 | 0.952 | 0.916 | 0.923 |
| Private alleles (all loci) | - | 27 | 15 | 14 |
| Uniqueness, $\varphi$ | - | ${ }^{3} 0.915$ | 0.568 | 0.564 |
| ${ }^{4}$ Gametic equilibrium | Yes $(P=0.448)$ | Yes $(P=0.086)$ | Yes $(P=0.695)$ | Yes $(P=0.135)$ |

${ }^{1}$ Excludes three $A$. alternata isolates from the NW quadrant of the USA.
${ }^{2}$ None of the maximum likelihood estimators of genotypic diversity were significantly different in any of the pair-wise combinations.
${ }^{3}$ A uniqueness of 0.915 implies that there is a $91.5 \%$ chance that an isolate containing a unique allele, relative to the meta-population, can be drawn from this subpopulation.
${ }^{4} P$-values indicate the probabilities of rejecting the null hypothesis of random association of alleles. A $P$-value of less than 0.05 is regarded as significant.

Table 4. Population differentiation $(\theta)$ and estimated number of allelic migrants per generation $(\hat{M})$ between the three artificial subpopulations of Alternaria alternata from the south-west, south-east, and north-east quadrants of the USA.

| Comparison | $\theta$ | $\hat{M}$ |
| :--- | :---: | :---: |
| NE-USA vs. SE-USA | 0.00506 | 98.33 |
| NE-USA $v s$. SW-USA | 0.01537 | 32.04 |
| SE-USA $v s$. SW-USA | 0.00338 | 147.50 |

## DISCUSSION

Alternaria species from section Infectoriae, the $A$. arborescens group and $A$. tenuissima (both section Alternaria) are described as common species from food and the indoor environment (Samson et al. 2010). Three species from the former genus Ulocladium, recently synonymized under Alternaria (Chapter 2), are also common in food and the indoor environment (Samson et al. 2010); Alternaria cucurbitae, A. atra (both section Ulocladioides) and A. alternariae (section Ulocladium). Our results largely support these observations for the indoor samples, although the species from section Alternaria were by far the most prevalent in the US homes included in this study. We only found one isolate from section Infectoriae and two isolates from section Pseudoulocladium, that resembles section Ulocladioides and Ulocladium based on morphology.

No correlation was found between the location and place of isolation and the eBURST groups assigned to the isolates based on their allelic profiles. Since most groups contained indoor
isolates as well as reference isolates, there did not seem to be a specific indoor cluster. However, there was subjective correlation between the eBURST groups and phylogeny; Alternaria gaisen, A. gossypina and $A$. burnsii isolates clustered together in both analyses. The other species that could be distinguished based on phylogeny, namely $A$. alstroemeriae, A. alternantherae, $A$. betae-kenyensis, $A$. iridiaustralis and $A$. longipes, were also separated using eBURST. The $A$. arborescens isolates did not form a single group based on their allelic profiles, but the isolates did cluster together in several eBURST groups ( $12,14,16$ and 18) or remained as singletons (6). Furthermore, 15 out of the 17 isolates from the $A$. arborescens species complex had allele 125 at locus AEM5 and allele 281 at locus AEM9. The two remaining isolates had one of the mentioned alleles but differed at the other locus. Although the A. iridiaustralis isolate also had these alleles, these loci have some potential as markers for species in the $A$. arborescens complex.

Surprisingly, analyses to test for random association of alleles in isolates of A. alternata showed that the allele associations between microsatellite loci were not significantly different from what can be expected in a randomly mating population. Nonetheless, alleles of AEM5 and AEM6, which were on the same locus, were in linkage disequilibrium. The last mentioned observation can be explained by the improbability of cross-over events between the two adjacent stretches of DNA. For these reasons, the less polymorphic of these two loci, i.e. AEM6, was excluded when disequilibrium was tested between loci.

Two possible explanations can be proposed for gametic equilibrium and, thus, outcrossing. The first is that cryptic sexual recombination could account for the lack of allelic associations. Evidence is accumulating for the occurrence of cryptic sex in filamentous fungi that are thought to be asexual (Kück \& Pöggeler 2009). For example, another study of an A. alternata population causing citrus brown spot in Florida revealed three subpopulations of which two were clonal and one showed the ability to recombine through a cryptic sexual cycle or parasexual cycle, based on six fast evolving loci and the presence of both mating-types (Stewart et al. 2013b). A second explanation for random association of alleles in $A$. alternata can be arrived at when we consider the nature of microsatellites. These loci change via birth-and-death evolution (Buschiazzo \& Gemmell 2006) such that they are highly polymorphic. It is possible that over long periods of asexual reproduction a microsatellite locus can become hyper-mutated in very large populations such as $A$. alternata. If this process acts equally on all microsatellites, such a situation could account for the random association of independently evolving alleles that were detected in this study. Thus, this explanation accounts for two possibilities: either the lack of allele association was due to experimental error (the inability of the available microsatellites to discriminate between randomly and non-randomly associated alleles), or A. alternata has been asexual for so long that the loci are hyper-mutated. A simulation of the observed data showed that sampling was adequate in both dimensions (i.e. number of isolates and number of loci). Additionally, due to size limitations on microsatellite loci (e.g. Buschiazzo \& Gemmell 2006) there is a very high probability of size homoplasy, confounding the detection of hyper-mutation. Therefore, recombination is the most parsimonious explanation for the data.

High levels of diversity can be caused only by a limited set of evolutionary processes. The most important of these are mutation, recombination, and migration (Ayala 1982, Hedrick 2000, Halliburton 2004; Hartl \& Clark 2007, Nielsen \& Slatkin 2013). Our data indicated that recombination is a contributor, but that hyper-mutation is not a viable explanation for the diversity of $A$. alternata. Although no subpopulations could be statistically identified, the levels of uniqueness provided important information regarding the movement of the fungus across the mainland USA. Since the SW-USA subpopulation was most unique, we can hypothesize
that either this subpopulation results directly from sexual reproduction, or the alleles have an alternate origin but are concentrated in this region.

The SW-USA and SE-USA subpopulations appear to exchange a very high number of interpopulation allelic migrants, and this pattern correlates with the anti-trade winds. Alternaria spores are known as dry air spores that are dispersed by wind (Andersen et al. 2012). Longdistance dispersal in the air can only occur if there is a susceptible host in the target area (Brown \& Hovmøller 2002). Since A. alternata has been described from more than 100 host plants (Rotem 1994), it is possible that these genotypes move through the air in a west-to-east direction across the southern USA. This is then possibly followed by south-to-north movement out of the SE-USA subpopulation towards to NE-USA. This long-distance movement of fungal spores from the southern USA to the northern USA has already been reported for the air-borne plant pathogens Puccinia graminis and Phakopsora pachyrhizi (Andersen et al. 2012).

The high genotypic diversity within the $A$. alternata isolates was also visible with our gene sequencing, as inconsistent sub-clusters existed within the three single-gene phylogenies. In a more extensive phylogenetic study on section Alternaria, where eleven individual gene regions were sequenced, the incongruent clustering within the A. alternata isolates was demonstrated even more clearly (Chapter 5). We speculate that this high genotypic diversity derives from Mexico / Central America, where many agricultural crops have evolved. From here the fungi moved through the USA via the antitrade winds.

## CONCLUSIONS

This study showed that the most prevalent species in the indoor environment in USA homes is A. alternata, with a high genotypic diversity. The SW-USA subpopulation displayed the highest level of uniqueness and the highest amount of gene flow, between SW-USA and SE-USA, coincided with prevailing winds over the subcontinent. Lastly, A. alternata in the continental USA displays random mating. This is the first report of such an observation in indoor samples of this fungus from homes in the USA.

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General discussion

## GENERAL DISCUSSION

The research presented in this thesis treats the taxonomic status of Alternaria and related genera with a further focus on the large-spored Alternaria species in section Porri and the small-spored Alternaria species in section Alternaria. Section Porri is the largest Alternaria section with regards to the number of species, and includes several important plant pathogens. The most commonly reported species in literature and type species of the genus Alternaria, Alternaria alternata, resides in section Alternaria. As A. alternata is considered as one of the most prolific producers of fungal allergens and is reported as pathogen on over 100 host plants, correct species identification is of utmost importance. In addition to the phylogenetic studies, genome and transcriptome sequence data were analysed to identify candidate loci for species identification within the small-spored Alternaria species, and microsatellite markers were used to study the genetic diversity and distribution of indoor Alternaria isolates in the USA. For end-users, the phylogenies and classifications presented in this thesis result in a more stable and understandable taxonomy and nomenclature of Alternaria and allied genera. This revised taxonomy will serve as a starting point for applied research performed by plant pathologists, breeders and medical mycologists in the field.

## From 14 genera to one

The results presented in this thesis radically changed how we perceive the genus Alternaria. By synonymizing 13 genera with Alternaria (Chapter 2), the morphological characters associated with the generic concept changed significantly. The question therefore arises: can one (still) identify Alternaria in the field based on morphology alone? Since almost all dematiaceous hyphomycetes with phaeodictyospores, which develop at a restricted site at the apex of distinctive conidiophores, are now called Alternaria, the identification becomes easier. No distinctions need to be made based on dark rigid septa (formerly Embellisia or Undifilum) or young obovoid conidia (formerly Ulocladium or Sinomyces). On the other hand, dematiaceous hyphomycetes with phragmospores without longitudinal septa (formerly Chalastospora or Nimbya), are now also classified as Alternaria. Even isolates which display cylindrical conidia (formerly Brachycladium) are now included in the genus, making the morphological generic description very broad. Although these morphological differences do not coincide with the synonymy of all these genera under Alternaria, the decision to synonymize them was based on i) a well-supported phylogenetic deeper node in multiple analyses, ii) limited sequence variation of clades within Alternaria (as defined in point i) based on SSU, LSU and ITS data, and iii) incongruent tree topologies for the clade order between the different single-gene phylogenies. Any alternative options, that are not supported by an underlying molecular phylogeny, would give rise to multiple genera consisting of only a few species each. This rise of a multitude of genera, potentially consisting of a small number of species each, would complicate the taxonomy even more, as several of these genera would have more or less identical morphological characters, which would make morphological identification in the field impossible.

Taking all these aspects into consideration, the description of a single all-encompassing Alternaria clade is the best solution for solving the confusion surrounding the taxonomy of Alternaria and allied genera and creating a stable and understandable taxonomy and nomenclature. By retaining the original genus names as new section names of Alternaria, the information linked to the former genus name is not lost.

## Genera unrelated to Alternaria

Besides the synonymy of several genera with Alternaria, the research presented in this thesis also provides molecular proof for the distinction of several genera from Alternaria. Five genera formerly linked to Alternaria are placed within the Pleosporaceae (Chapter 2). The asexual genus Stemphylium, with a Pleospora sexual morph, forms a clade distinct from the Alternaria clade (Chapter 2). Morphologically the genus can be distinguished from Alternaria based on the percurrent proliferation of its conidiophores, while Alternaria displays a geniculate, sympodial proliferation (Chapter 1, Fig. 1). The sexual genera Comoclathris and Clathrospora were described within the Pleosporaceae with alternaria-like asexual morphs (Zhang et al. 2011). Both genera were previously placed within the Diademaceae, a family which is characterised by a flat circular lid as ascoma opening and applanate, bitunicate asci (Shoemaker \& Babcock 1992). The sexual morphs of Alternaria (Lewia, Allewia and Crivellia) segregate from a heterogenous group of species with relatively small ascomata, historically placed in Pleospora. They form a central pore in a terete cylindrical beak as ascomatal opening and form subcylindrical, bitunicate asci.The results in this thesis show that both Comoclathris and Clathrospora belong to the Pleosporaceae, but are clearly unrelated to Alternaria (Chapter 2), as the morphological characters of the sexual morphs already suggested. Also the newly described Paradendryphiella, which accommodates the marine Dendryphiella species, D. arenariae and D. salina (= Embellisia annulata), forms a clade distinct from Alternaria within the Pleosporaceae (Chapter 2). Morphologically they can be distinguished from Alternaria based on the prominent conidial scars on the conidiogenous cells, and the narrow denticles the conidia are produced on.

Based on molecular data, the genus Alternariaster, described to accommodate the former Alternaria helianthi (Simmons 2007), was placed within the Leptosphaeriaceae, instead of the Pleosporaceae to which Alternaria belongs (Chapter 2). The initial segregation from Alternaria was based on morphological characters alone (Simmons 2007). From the three genera which were simultaneously segregated from Alternaria based on morphology (Simmons 2007), the other two genera, Chalastospora and Teretispora, were reduced to synonymy with Alternaria after molecular studies (Chapter 2). For Alternariaster and Prathoda, a rejected genus that was revisited, molecular data supported the morphological segregation from Alternaria and, after molecular study, both genera ended up in a different family to Alternaria (Chapter 2, Pryor \& Gilbertson 2000). The discovery of a second species in the genus Alternariaster resulted in a reappraisal of the genus (Chapter 3).

With the data presented here, the confusion surrounding the above-mentioned six genera and their relation to Alternaria is clarified. Unfortunately it was not possible to obtain living cultures from the genera Briansuttonia and Rhexoprolifer, which are also linked to Alternaria (Seifert et al. 2011). These genera still lack molecular data, and their phylogenetic relation with Alternaria presently remains unresolved. The recollection of fresh material representing these genera would resolve the last unresolved aspect pertaining to the taxonomy of genera with muriformly septate conida.

## From species-group to section

The genus Alternaria has a long history of being divided into species-groups based on morphology (Chapter 1). These species-groups were mainly based on conidium morphology and sporulation pattern, e.g. small- or large-spored, conidia with or without beaks, conidia
single or in chains, simple or in branched chains. Numerous molecular studies revealed that Alternaria species cluster in distinct species clades, but these are not always correlated with the species-groups based on morphological characteristics alone. Before the start of this PhD study, seven species-groups were recognised based on molecular phylogenetic data (Chapter 1). During the present study, the species-group concept was formalized by introducing sections in Alternaria (Lawrence et al. 2013). In the International Code of Nomenclature for algae, fungi and plants, the Melbourne code (ICN; McNeill et al. 2012), a section is an officially recognized taxonomic rank below the genus and above species level. This is in contrast to the term speciesgroup, which has no official taxonomic status. Sections are mainly used to help organise very large genera. Initially, eight well-supported "asexual" lineages of Alternaria were elevated to the taxonomic rank of section, based on a phylogenetic study of five genes (Lawrence et al. 2013). In addition to six known species-groups, now named section Alternantherae, section Alternaria (previously A. alternata species-group), section Brassicicola, section Porri, section Radicina and section Sonchi, two new lineages were introduced, namely section Gypsophilae and section Panax. The "sexual" A. infectoria species-group was not included in the sections, since it was not phylogenetically supported as Alternaria. The asexual / sexual division mentioned in the manuscript introducing the sections within Alternaria (Lawrence et al. 2013), was not supported in the dataset presented in this thesis, as two sexual forms were placed in section Panax (Chapter 2).

With the synonymy of 13 genera under Alternaria, the introduction of sections by Lawrence et al. (2013) was extended over the entire genus, resulting in 24 Alternaria sections and six monotypic lineages. The $25^{\text {th }}$ section, section Euphorbiicola, was introduced when studying section Porri (Chapter 4), and the $26^{\text {th }}$ section, section Soda (Grum-Grzhimaylo et al. 2015) followed soon thereafter. Two species in a newly described genus, Pseudoalternaria, can most likely also be assigned to a new section of Alternaria. During the same time-period as the introduction of the big Alternaria clade (Chapter 2), this new genus was introduced for two species closely related to the $A$. infectoria species-group (Lawrence et al. 2014). Since the publication of this new genus (accepted 3 June 2013, published online 22 June 2013) coincides with the publication that synonymized the 13 genera under Alternaria (Chapter 2, published online 31 May 2013), the genus Pseudoalternaria was already reduced to synonymy under Alternaria before the publication appeared. It is interesting to note that the authors describing Pseudoalternaria earlier suggested a further taxonomic revision of the $A$. infectoria speciesgroup to eliminate the polyphyly within Alternaria (Lawrence et al. 2013). However, their later published study on the $A$. infectoria species-group (Lawrence et al. 2014) did not deal with the mentioned polyphyly, but only introduced this new genus, Pseudoalternaria, which applied to two isolates that cluster just outside the $A$. infectoria species-group.

When looking at the currently described sections, some sections correspond with the morphologically based species-groups, like section Alternaria, section Porri and section Infectoriae. However, other sections display a more diverse morphology, but seem to have a biological binding factor, like section Gypsophilae and section Japonica which seem to be linked to a certain host family, respectively Caryophyllaceae, Brassicaceae; and section Phragmosporae which seems to relate to the environment in which it occurs, namely soil and seawater environments. In other words, some sections contain similarly looking species, which occur on multiple host families, while other sections contain species which occur on specific host families, or environmental conditions, but vary in morphology. More study is necessary to try to explain why some molecular and morphological related species are able to spread to different hosts, while other molecularly related species evolve within a certain host family.

Although the synonymy of multiple genera with Alternaria gave rise to a very large genus, with hundreds of described species, the introduction of sections within the genus makes the taxonomy and nomenclature of the genus manageable. With a GAPDH sequence alone it is relatively easy to identify to which Alternaria section an isolate belongs. With this knowledge, one can focus on the species assigned to the appropriate section, which reduces the complexity of the identification significantly.

## Alternaria section Porri

The largest Alternaria section, in terms of the number of species, is Alternaria section Porri. The species in this section are characterized by their medium to large conidia with a long (filamentous) beak. Among them are numerous important plant pathogens, such as $A$. porri, A. solani, and A. tomatophila. The research presented in this thesis (Chapter 4) treats all 82 known morphospecies within this section. Based on a five-gene phylogeny, combined with morphology, only 63 species are supported within section Porri. Twenty-seven species are placed in synonymy and from the 63 supported species, 10 are newly described. The phylogeny gives a complete overview of the species within the complex and their closest relatives, and the host where they were isolated from.

Most morphospecies described in section Porri were presumed to be host-specific. The present study reveals that most species are at least host family-specific ( 49 out of 63 species), but eight species are found on two different host families, and six species on three or even more host families (Chapter 4, Table 2). Interestingly, the species with the broadest host range, $A$. solani-nigri, found on five different host families, seems to have a geographical preference. All A. solani-nigri isolates included in this study originate from New Zealand, although the taxon was originally described from India. Also the pathogens originally known from Solanaceae seem to possess a broad-host range. Following the concept of Ellis (1971), almost all largespored, narrow-beaked Alternaria strains hitherto isolated from Solanaceae were called A. solani. A morphological distinction was later made between tomato (A. tomatophila, A. cretica, A. subcylindrica) and potato (A. solani, A. grandis) pathogens (Simmons 2000), which was supported by subsequent molecular studies and chemotaxonomy (Andersen et al. 2008, Rodrigues et al. 2010, Brun et al. 2013, Gannibal et al. 2014). This distinction between tomato and potato pathogens is confirmed here, although the tomato pathogens are now synonymized into one species, to which the oldest name $A$. linariae applies. However, these Solanaceae pathogens include multiple isolates collected from different host families, which suggest a broad host range. Also A. protenta, formerly known as a Helianthus annuus-specific pathogen ( $\mathrm{Wu} \& \mathrm{Wu} 2003$ ), which now contains an isolate collected as an early blight pathogen of Solanum tuberosum (originally described as $A$. solani), is shown to be present on multiple hosts. Follow-up studies on these presumably broad host-range species could demonstrate whether the separate isolates within these species can really infect multiple hosts, or whether there are different host-specific lineages within the species, such as which are present in Alternaria section Alternaria (Chapter 5).

## Alternaria section Alternaria

The section with the biggest confusion with regards to correct identification is by far Alternaria section Alternaria, since species are mostly based on morphology and host-specificity, even though the molecular variation is minimal. This section contains Alternaria species with small
conidia formed in chains, which are the most common Alternaria species found throughout the world. They are mostly saprophytic, but can become pathogenic when their surroundings change. For instances as post-harvest pathogen, when fruit gets damaged and the fungus enters the underlying tissue under the right conditions. The same applies to phaeohyphomycosis in humans, when the fungus gets the opportunity to enter the mammalian tissues, for instance with a splinter, they can proliferate under the right conditions and cause infection. This is especially true with a decreased or suppressed immune system, as present in immuno-compromised patients. Furthermore, it is known that Alternaria species from section Alternaria can acquire a small dispensable chromosome, which contains a host-specific toxin gene cluster (Salamiah et al. 2001, Masunaka et al. 2005, Akagi et al. 2009). This will enable the fungus to proliferate on the host the toxin acts on, which it was not able to do without the obtained gene cluster.

Based on genome-sequencing combined with transcriptome profiling and multi-gene phylogeny the research performed in this thesis (Chapter 5) reduces the species in this section from 52 morphospecies to only 11 phylogenetic species and one species complex. Thirty-five species are synonymized under A. alternata, of which one, Alternaria viniferae, was just recently described (Tao et al. 2014). Some clustering in clades is observed for isolates in the different single-gene phylogenies, based on minor nucleotide changes, but the observed clades are incongruent between the different single-gene phylogenies for the A. alternata isolates. No two genes show the same clustering, and isolates from one fully supported clade in one gene-tree, will cluster with isolates in other clades in another single gene-phylogeny, and are found on different places again in a third gene tree. The same applies to phylogenies derived from two new genes, which were identified as candidate phylogenetic markers based on promising "low" conservation values found when comparing the sequenced Alternaria genomes (Chapter 5). The L152 gene, which was suggested as a potential key for discriminating the small-spored Alternaria species (Roberts et al. 2012), was also tested. However, even with the L152 sequence alignment, the phylogenetic tree did not correlate with other single gene trees, and it could not distinguish all recognized species within section Alternaria (Fig. 1). In the L152 phylogeny A. alternata isolates are divided into several clusters, with other recognized species clustering among them. Alternaria burnsii cannot be distinguished from several $A$. alternata isolates, and one isolate from the $A$. arborescens species complex (AASC) clusters separately (Fig. 1).

The incongruencies between the single-gene trees in combination with a high similarity with the genome comparison and transcriptome profiles, led to the conclusion to synonymize most of the morphospecies within section Alternaria. The incongruencies in the single-gene phylogenies (Chapter 5), and the high diversity seen with the microsatellite analysis of indoor A. alternata isolates (Chapter 6), demonstrate that $A$. alternata is a species with a high genotypic diversity. Furthermore, the population genetic study on the indoor A. alternata population showed that they display random mating in the USA, suggesting a sexual cycle (Chapter 6). This fits with the high diversity found among isolates and the incongruencies in the single-gene phylogenies, which would be difficult to explain in a clonal / asexual population.

With the aid of the provided sequence-based identification guide (Chapter 5), the species identification in this section now becomes much clearer and easier. However, when one has identified an isolate as $A$. alternata, the next question one should ask is about the presence or absence of a dispensable chromosome, and whether it contains one or even multiple hostspecific toxin genes. The presence or absence of a toxin cluster defines whether an $A$. alternata isolate is capable of acting as a true plant pathogen or probably only acts as an opportunistic pathogen.


Fig 1. Bayesian $50 \%$ majority rule consensus tree based on the L152 gene sequence alignment. The Bayesian posterior probabilities (PP) are given at the nodes; thickened lines indicate a PP of 1.0. Isolates from the same species are indicated with blocks of the same colour. AASC $=A$. arborescens species complex. The tree was rooted with $A$. avenicola CBS 121459.

## Indoor Alternaria

Alternaria is known as an important air-borne allergen causing hypersensitivity reactions in humans, which can eventually lead to asthma (Downs et al. 2001). The allergens responsible for these hypersensitivity reactions are located on the cell wall of the (asexual) conidia (Twaroch et al. 2012). Since Alternaria is commonly found outdoors, little study has been performed on indoor Alternaria populations. Also the difficulties in identifying species within section Alternaria, to which section the main airborne allergen A. alternata belongs, hampered the research performed on indoor Alternaria populations. The present study (Chapter 6) of an extensive indoor Alternaria population collected throughout the USA, shows that $98 \%$ (153 isolates) of the indoor isolates indeed belong to section Alternaria. After species identification (as described in Chapter 5), $88 \%$ (137 isolates) belonged to $A$. alternata, which was previously reported to be the main airborne allergen, and is, as shown, also the most prevalent species within the indoor environment. The present study further shows that based on microsatellite data, the indoor Alternaria isolates collected throughout the USA do not form a specific indoor cluster (Chapter 6). This refutes the assumption that indoor air Alternaria species are specially adapted to the environment they live in.

## Genome versus (multi-)gene phylogenies

Study of the genomes of representatives of seven different sections described within Alternaria (Chapter 5) shows an identical topology to the multi-gene studies (Chapter 2). Not every mycologist and pathologists embraced the synonymy of all (well-known) genera under Alternaria, although the molecular data does not leave much room for other sensible conclusions (Chapter 2). It was already stressed earlier in the Discussion that this was the only logical choice to make, in order to establish a stable and understandable taxonomy and nomenclature. The research was presented as posters and oral presentations at several occasions to an applied / end-user public; this resulted in discussions with numerous mycologists and plant pathologists, who mostly agreed with the presented findings and welcomed the clarity that it provides. However, there is still a small group of researchers who could not bring themselves to agree with the presented findings, although they can neither come up with strong arguments for their disagreement, nor with a (better) solution for this taxonomic problem. In order to provide additional support for the presented findings, a phylogenetic tree was constructed on whole genome sequence data of the newly sequenced Alternaria spp., together with the genomes of closely related genera. The program REALPHY (Chapter 5) was used to compare the new genomes with the sequenced genomes from the closest related genera publicly available from the Joint Genome Institute (http://jgi.doe.gov/). We included the genomes from the closely related genera Bipolaris and Curvularia, together with two Pyrenophora genomes and one Exserohilum genome. To be able to create a reliable REALPHY phylogeny, the genera should not be phylogenetically too far apart, since this would leave too little genome data with homology to compare. The taxonomic status of the genera Bipolaris and Curvularia was also under debate, since they were both linked to the same sexual morph Cochliobolus (Manamgoda et al. 2012). However, recent studies confirmed that they are indeed two separate genera (Manamgoda et al. 2014, Ariyawansa et al. 2015). These genera therefore form the perfect test case for the included Alternaria panel. In the resulting phylogenetic tree (Fig. 2), a clear vertical species line can be drawn, which separates all genera included in the phylogeny, and which supports all the species


Fig 2. PhyML tree based on the whole genome and transcriptome reads of 20 isolates using REALPHY, including 195205 nucleotide positions. The bootstrap support values $>60$ are given at the nodes; thickened lines indicate a fully supported node. The tree was rooted following a phylogenetic study of the Dothideomycetes (Schoch et al. 2009).
within the genus Alternaria. This further confirms the decision to establish one encompassing Alternaria genus concept containing numerous sections, and should convince most of the remaining sceptic mycologists and plant pathologists to adopt the approach highlighted in this thesis.

## General conclusions

This study started off with The Alternaria Identification Manual (Simmons 2007), and the underlying cultures linked to the species in the Manual, as basis. Multi-gene sequence data were generated, which were used for accurate species identification. During this study, the concepts used for species identification by Simmons (2007) could be confirmed for numerous species, whereas others were shown to be synonymous. The generic concept of Alternaria was revised and the use of the taxonomically informative sections favoured. The large-spored Alternaria species with long beaks, residing in section Porri, were disentangled and some species were confirmed to be host-specific whereas the host ranges for others were expanded. The identification of the small-spored Alternaria species from section Alternaria was clarified and, by providing a sequence-based identification guide, made available to a broader public. Furthermore, it has also been shown that the most common indoor Alternaria species in the USA is $A$. alternata, which seems to proliferate sexually, and does not form a specific indoor cluster.

The fundamental work performed in this thesis will provide plant pathologists, breeders and medical mycologists in the field the essential basis for the applied research they plan to perform. The coming years the implications of this dissertation's findings to the medical and agricultural field will need to be tested. This thesis forms the basis for a new era in Alternaria research.

## APPENDIX

## APPENDIX

REFERENCESSUMMARYACKNOWLEDGEMENTSCURRICULUM VITAELIST OF PUBLICATIONS
EDUCATION STATEMENT

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## SUMMARY

The omnipresent dematiaceous hyphomycete genus Alternaria is associated with a wide variety of substrates including seeds, plants, agricultural products, humans, soil and even the atmosphere. It includes saprophytic, endophytic and pathogenic species, among which multiple plant pathogens, post-harvest pathogens, and human pathogens (causative agents of phaeohyphomycosis and hypersensitivity reactions). Molecular studies reveal that the Alternaria complex comprises nine genera; Alternaria, Chalastospora, Crivellia, Embellisia, Nimbya, Stemphylium, Ulocladium, Undifilum and Sinomyces. Within this complex several genera are non-monophyletic and Alternaria species cluster into multiple distinct species clades, which are not always correlated with speciesgroups based on morphological characteristics. The most commonly reported species in literature and type species of the genus Alternaria, A. alternata, also comprises one such a species-group. The small-spored Alternaria species within this group are mainly described based on morphology and / or host-specificity, but are difficult to distinguish based on molecular techniques alone. As A. alternata is considered as one of the most prolific producers of fungal allergens and is reported as pathogen on over 100 host plants, correct species identification is of utmost importance. The research presented in this thesis discusses the taxonomic status of Alternaria and its related genera, with a further focus on the two biggest and most important species complexes; the large-spored $A$. porri and small-spored $A$. alternata species complexes. With the phylogenies and classifications presented in this thesis, more robust and understandable taxonomy and nomenclature in Alternaria and allied genera within the Alternaria complex are created.

Chapter 1 gives a general introduction to the genus Alternaria and related genera. The history of the genus and its economic importance as plant pathogen, post-harvest pathogen, causative agent of phaeohyphomycosis and common allergen causing hypersensitivity reactions are summarized. The introduction of the morphological species complexes, based on characters of the conidia, the pattern of chain formation, and the nature of the apical extensions of conidia are treated. These morphological species-groups do not always correlate with molecular species-groups. Molecular studies recognise seven Alternaria species-groups, within the Alternaria complex; A. alternantherae, A. alternata, A. brassicicola, A. infectoria, A. porri, A. radicina and A. sonchi. Besides Alternaria, eight other genera are assigned to the Alternaria complex based on molecular and morphological studies; Chalastospora, Crivellia, Embellisia, Nimbya, Stemphylium, Ulocladium, Undifilum and Sinomyces.

Chapter 2 focusses on the relationship of Alternaria and its closely related genera within the broader Alternaria complex. The phylogenetic lineages within the Alternaria complex are delineated based on nucleotide sequence data of parts of the 18 S nrDNA (SSU), 28 S nrDNA (LSU), the internal transcribed spacer regions 1 and 2 and intervening 5.8S nrDNA(ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), RNA polymerase second largest subunit (RPB2) and translation elongation factor 1-alpha (TEF1) gene regions. The phylogenetic data reveal a Stemphylium clade sister to Embellisia annulata and a big Alternaria clade. The Alternaria clade contains six monotypic lineages and 24 internal clades, which are treated as sections of Alternaria. In order to create a stable phylogenetic taxonomy, and supported by i) a well-supported phylogenetic node in multiple analyses, ii) a high-similarity of clades within Alternaria based on SSU, LSU and ITS data, and iii) variation in the clade order between the different gene phylogenies, 13 genera (Allewia, Brachycladium, Chalastospora, Chmelia, Crivellia, Embellisia, Lewia, Nimbya, Sinomyces, Teretispora, Ulocladium, Undifilum and Ybotryomyces) are placed into synonymy
with Alternaria. When applicable, the former generic names are retained but now with a different taxonomic status as section name. Embellisia annulata is synonymized with Dendryphiella salina, and together with D. arenariae placed in the new genus Paradendryphiella. The sexual genera Clathrospora and Comoclathris, with asexual forms linked to Alternaria, cluster within the Pleosporaceae, as does Alternaria, but outside Alternaria s. str. The genus Alternariaster, described to accommodate Alternaria helianthi, clusters within the Leptosphaeriaceae.

Chapter 3 describes the reappraisal of the genus Alternariaster. Alternaria helianthi, the causal agent of leaf spot on Helianthus annuus (sunflower) was segregated from Alternaria based on conidial morphology, and placed in the new genus Alternariaster. A molecular study confirmed the segregation from Alternaria (Pleosporaceae), and placed the genus in the Leptosphaeriaceae. A multi-gene phylogeny of parts of the ITS, LSU, RPB2 and GAPDH gene regions placed a fungal pathogen associated with leaf spot on Bidens sulphurea (yellow cosmos) in Brazil in close relation with Alternariaster helianthi. Morphologically the newly found pathogen has smaller conidia to Al. helianthi, and lacks oblique or transverse septa, which can be present in $A l$. helianthi. Pathogenicity studies on 18 plant species from the family Compositae showed that the newly found fungus was only able to infect $B$. sulphurea, whereas $A l$. helianthi could infect $H$. annuus and Galinsoga quadriradiata, a yet unreported host of Al. helianthi. Based on the close phylogenetic relation to Al. helianthi, but distinct morphological and pathogenicity characters, the fungal pathogen associated with leaf spot on B. sulphurea is newly described as Al. bidentis.

Chapter 4 treats the Alternaria species which form the largest section of Alternaria, section Porri. This section contains almost all Alternaria species with medium to large conidia with long beaks, some of which are important plant pathogens. A multi-gene phylogeny on parts of the ITS, GAPDH, RPB2, TEF1 and Alternaria major allergen (Alt a 1) gene regions, supplemented with morphological and cultural studies, forms the basis for species recognition in this section. The polyphasic data reveal 63 species in section Porri, of which 10 are newly described, and 27 names are synonymized. The three known Alternaria pathogens causing early blight on tomato, A. cretica, A. subcylindrica and A. tomatophila, are synonymized under the older name $A$. linariae. A third pathogen, A. protenta, is reported as the cause of early blight of potato, next to $A$. solani and $A$. grandis. Alternaria protenta was formerly known as pathogen of $H$. annuus, but is here reported from hosts from four different plant families. Next to the known pathogen on sweet potato, $A$. bataticola, three more species are delineated, of which two are newly described as $A$. ipomoeae and $A$. neoipomoeae. Two clades with isolates causing purple blotch on onion are confirmed as $A$. allii and $A$. porri, but they cannot adequately be distinguished based on their numbers of beaks and branches as described previously. The Passifloraceae pathogen $A$. hawaiiensis is synonymized under $A$. passiflorae, although it was described as lacking multiple beaks, which is a characteristic of $A$. passiflorae. From the data of these pathogens on onion and the Passsifloraceae, it can be deduced that the number of beaks does not seem to be a valid morphological character for species differentiation within section Porri.

Chapter 5 treats the small-spored Alternaria species, which reside in section Alternaria. A lot of confusion around the naming of species within this section exists, since the naming is mostly based on morphology and host-specificity, although the molecular variation is minimal. Whole genome sequencing, combined with transcriptome profiling and multi-gene sequencing of nine gene regions, SSU, LSU, ITS, GAPDH, RPB2, TEF1, Alt a 1, endopolygalacturonase (endoPG) and an anonymous gene region (OPA10-2), is used to create a clear and stable species classification in this
section. The nine sequenced Alternaria genomes, A. alternantherae, A. alternata, A. arborescens, A. avenicola, A. brassicicola, A. citriarbusti, A. gaisen, A. infectoria, A. papaveraceae, A. solani, and $A$. tenuissima, range in size from $32.0-39.1 \mathrm{Mb}$. The number of repetitive sequences varies significantly, with a relatively low percentage of repeats within section Alternaria. The genome identity within section Alternaria is high, compared to the genome identity for isolates from other sections to the $A$. alternata reference genome. Similarly, a relatively low percentage of single nucleotide polymorphisms (SNPs) were observed in genomic and transcriptomic sequences between isolates from section Alternaria, compared to the percentage of SNPs found in isolates from different sections compared to the $A$. alternata reference genome. The topology of a phylogenetic tree based on the whole-genome and transcriptome reads was congruent with multigene phylogenies based on commonly used gene regions. A set of core proteins was extracted from the genome and transcriptome data, and primers were designed on two eukaryotic orthologous group (KOG) protein loci with a relatively low degree of conservation within section Alternaria. The phylogenies from these two gene regions, KOG1058 and KOG1077, could not distinguish the described morphospecies within section Alternaria better than the phylogenies based on the nine commonly used gene regions for Alternaria. Based on genome and transcriptome comparisons and molecular phylogenies, Alternaria section Alternaria consists of only 11 phylogenetic species and one species complex. Thirty-five morphospecies, which cannot reliably be distinguished based on the multi-gene phylogeny, are synonymized under $A$. alternata. The subclades that are formed by these isolates are incongruent between the different gene regions sequenced; no two genes show the same groupings for any of the over 100 isolates. A sequence-based identification guide is provided for the species which are now recognized in section Alternaria. None of the genes sequenced in this study can distinguish all of the species recognized here on its own.

Chapter 6 investigates the molecular diversity of indoor Alternaria isolates in the USA, with the help of a phylogeographic / population genetic approach. Isolates collected throughout the USA were identified using ITS, GAPDH and endoPG gene sequencing, followed by genotyping and population genetic inference of the section Alternaria isolates and 37 reference isolates, using five microsatellite markers. Phylogenetic analyses revealed that $98 \%$ ( 153 isolates) of the indoor isolates consisted of species from Alternaria section Alternaria. The remaining $2 \%$ (three isolates) represented one section Infectoriae and two section Pseudoulocladium isolates. From the 153 isolates that belonged to section Alternaria, one could be assigned to A. burnsii, 15 to the $A$. arborescens species complex and the remaining 137 isolates were identified as $A$. alternata. Based on the microsatellite data, no specific indoor population could be distinguished. However, the microsatellite data did correlate with the phylogenetic data. Population assignment analyses of the $A$. alternata isolates suggested that subpopulations did not exist within the sample, which we thus divided into four artificial subpopulations to represent four quadrants of the USA. Genotypic diversity was extremely high for all quadrants and a test for linkage disequilibrium suggested that $A$. alternata has a cryptic sexual cycle. The SouthWest-USA population displayed the highest level of uniqueness, based on private alleles. Intriguingly, the highest amount of gene flow, between SouthWest-USA and SouthEast-USA, correlated with the west-to-east movement of the antitrade winds. This suggests that indoor A. alternata isolates, although extremely diverse, have a continental distribution and high levels of gene flow over the continent.

Chapter 7 discusses the data presented in this thesis. The implications of the performed studies are placed in a broader context, with a focus on the relation between morphology and the new species classification based on molecular tools and the use of genome data in contrast to multi-gene data.

## ACKNOWLEDGEMENTS

Where to start? That is easy! There is only one person who really made this thesis possible, and that is my promotor Pedro Crous. Pedro, many thanks for believing in me, and the opportunity you gave me to start my own PhD project in your group at the CBS-KNAW. You had asked me earlier to start my own PhD project, but since I had already planned to start a family I thought a PhD would not fit that picture. However, after the birth of my first daughter, you still believed I would be able to successfully finish a PhD , and asked me the same question again. It was never my intention to pursue a PhD degree, but I did always regret that I did not go to University, and I loved to perform research, although I was not fond of having to give oral presentations. With the support and encouragements of my parents and partner, I decided to grab the chance you gave me. I hope the completion of this thesis will not be the end of us working together, and that we will publish many more nice manuscripts together.

Next to Pedro, I of course want to thank Pierre de Wit, my other promotor, for his critical comments, and valuable input he made during this study. I also want to thank my co-promotor and daily supervisor Ewald Groenewald, and when Ewald wasn't available, Lorenzo Lombard, for all their help with my research. Both of you always took the time to help me wherever you could. I would also like to mention Lute-Harm Zwiers, who unfortunately left the CBS-KNAW when I just started my PhD, but taught me a lot, while working on the Phoma / Ascochyta mating-type article together. Lute-Harm, thank you for everything. Of course I cannot forget to mention Hans de Gruyter (nVWA NPPO-NL, Wageningen), for whom I performed the lab work during his part-time PhD study. He showed me how a PhD worked, and as his "paranimf" I could even see how the actual PhD defence goes in practice. Hans, thank you for the nice cooperation, and I wish you all the best at nVWA and with your family. I am sure we shall keep in contact. When I refer to Hans, I also have to mention Maikel Aveskamp, the other PhD student working on Phoma at the same time. Since Hans was only at the CBS one day a week, I mostly worked together with Maikel in the lab. Maikel, thank you for the collaboration and good luck with your career and your lovely family. Back to the present, I want to thank Michael Seidl (Laboratory of Phytopathology, WUR), who helped / helps me with the genome work. Michael, thank you for all our discussions, and your sincere interest in my progress. I am sure our collaboration will not end here, and I hope we shall be able to publish some (more) nice manuscripts together. I would also like to thank Sandra Videira, who started her PhD in our research group at the same time period as I did. Sandra, thank you for all the conversations on our PhD projects and daily topics, and for being my "paranimf". I wish you good luck with the final part of your PhD and all the best with the rest of your career and future life with Luís. To William Quaedvlieg, the former PhD student from our group and room-mate in the PhD room, thank you for all the talks and discussions, and wish you the best with your career. To my other (current) room-mates, Tao Yang and Xuewei Wang, I wish you all the best with your PhD, and Chaetomium work, respectively.

I also want to mention the technicians in our group, Mieke Starink and Arien van Iperen, thank you both for the nice conversations and help when necessary. And of course, Mieke, thank you for being my other "paranimf". A special thanks to Janneke Bloem, who helped me with isolating DNA and performing PCR amplifications while she was working as a technician in our group. Janneke, good luck with your career at the NIOO, and hope to see you again. There are two German postdocs that previously worked in our research group whom I would like to mention, Ulrike Damm and Manfred Binder, thank you both for sharing your knowledge with me. I really appreciated the conversations with both of you. I shall not mention all the guests
who visited our group while I worked here the last 8 years, first as technician and then as PhD student, because I am sure I will forget some of them. But if you feel addressed, thank you for all the nice conversations! Also the staff from the Collection, thank you for the support and nice small talks, although I did not join you at the coffee table that much anymore after starting my own PhD project. Two persons whom I do want to mention by name are Marjan Vermaas and Manon Verweij. Marjan, thank you for all the help with taking pictures and creating beautiful photo plates and all the interest you showed in my PhD progress. Manon, I would like to thank you for all the help you gave me with the lay-out of the manuscripts, and eventually this thesis, and all the other help you ever gave me. It was a pleasure to get to know you a bit better during the congress in Thailand. Finally a general thank you to all other scientific and supporting staff at the CBS-KNAW, who made my stay at the CBS-KNAW a pleasant one.

Before thanking the persons who supported me in my personal life, I would like to take the opportunity to thank one more person who stimulated me in my career. Paul Savelkoul was my supervisor while working at the VUmc. Although the job mainly consisted of diagnostic work, he stimulated me, and gave me the opportunity to work on small research projects alongside my daily work. Paul, with your support I could really develop my love for performing research, which now eventually resulted in obtaining my PhD degree. Thank you for your trust.

Finally I would like to thank my family, whose support enabled me to finish my PhD study. I want to highlight some of them, but I will do this in Dutch.

Allereerst mijn ouders, Sjaak en Herma Woudenberg. Toen ik aan dit project begon, heb ik hen gevraagd of ze mij waar nodig wilden helpen met de opvang van de kinderen. Pap en mam, het was heerlijk om altijd op jullie terug te kunnen vallen, en ons totaal geen zorgen te hoeven maken om de meiden. We wisten dat er goed voor ze werd gezorgd en dat ze het prima naar hun zin hadden. Helaas gooide papa zijn ziekte de boel behoorlijk in de war, maar gelukkig gaat het alweer een stuk beter. Hopelijk kunnen jullie samen nog lang genieten van het leven en de kleinkinderen. Nogmaals ontzettend bedankt voor al jullie steun. Dan wil ik ook mijn schoonmoeder Willeke Piket en schoonzussen Barbara en Yvette Piket bedanken. Wanneer we toch in de knoei zaten met de meiden konden we altijd nog op jullie terugvallen, bedankt hiervoor! Ook wil ik graag mijn schoonvader nog even noemen, John Piket, die helaas mijn promotie niet meer mee zal maken. John was altijd oprecht geïnteresseerd in mijn onderzoek en, zo hoorde ik later, was zelfs bereid geweest om mij te vergezellen naar Amerika zodat ik niet alleen naar mijn eerste congres zou hoeven. Ik weet zeker dat hij trots op me zou zijn, John, we zullen je nooit vergeten. En dan uiteraard mijn partner Roy Piket. Roy, zonder jouw steun was dit zeker niet mogelijk geweest. Vooral de keren dat ik een week op pad moest voor het bijwonen van een congres en jij, met wat hulp van mijn ouders, zonder klagen of problemen de zorg voor onze twee meiden op je nam. Zelfs toen Demi pas vijf maanden oud was, en we ook nog een twee-is-nee peuter rond hadden lopen. Bedankt dat je mij vanaf het begin hierin voor de volle $100 \%$ hebt gesteund en geen moment hebt getwijfeld of ik dit wel zou kunnen. Ik heb mij wel meerdere keren afgevraagd of je wel helemaal door had waar ik aan begon, maar uiteindelijk heb je wel gelijk gekregen. Als laatste lijkt het mij op zijn plaats om nog mijn excuses te maken tegenover mijn fantastische, lieve, eigenwijze meiden Quinty en Demi Piket. Sorry meiden, voor de keren dat mama er niet was, lichamelijk of geestelijk, maar ook ontzettend bedankt voor alle afleiding die jullie mij gaven. Hoewel ik toch stiekem toe moet geven dat ik soms ook blij was met de afleiding die mijn werk mij gaf van alle baby / peuter / kleuter problemen. Ik ben nu al ontzettend benieuwd hoe jullie toekomst eruit gaat zien!

## CURRICULUM VITAE

Joyce Woudenberg was born on July $15^{\text {th }} 1980$ in Cothen, The Netherlands. In 1998, after she obtained her VWO-diploma at the Revius Lyceum in Doorn, she started with a HLO-study at the Hogeschool Utrecht. Here she fulfilled a diagnostic internship at the microbiology laboratory of the UMC Utrecht, and a research internship on the typification of Legionella isolates from surface water at Kiwa N.V. Water Research. She obtained her HLO-diploma in the Medical Microbiology in 2002.

After graduation she started as a research technician at the NCCB (Dutch Culture Collection of Bacteria), part of the CBS-KNAW, on a project funded by the Dutch ministry VROM, now called Ministry of Infrastructure and the Environment. The project aimed at confirming the identity of genetically modified organisms from Dutch laboratories. Besides this project she helped maintaining the bacterial collection, performed bacterial identifications for external applicants and wrote ISO-protocols for the upcoming ISO-certification of the CBS-KNAW Collection. Due to a reorganization at the CBS-KNAW in 2004, she had to search for a new challenge, which she found at the VU Medical Center in Amsterdam. Here she worked in the Molecular Epidemiology (MEP) group, part of the Medical Microbiology and Infection prevention (MMI) department. The MEP focused on the typification of bacteria, mainly with Amplified Fragment Length Polymorphism, to detect and control hospital infections. She was also trained to work in the Virology, Serology and Molecular Diagnostic groups. In addition to the diagnostic work, she got the opportunity to work on multiple small research projects. The MEP group was located at the university building, together with the research group of the MMI department; this stimulated her love of performing research.

In 2007 she got the opportunity to return to the CBS-KNAW, this time in the Evolutionary Phytopathology group of Pedro Crous. As a research technician she assisted two PhD students with unraveling the fungal genus Phoma and allied genera, and other PhD students and postdocs when necessary. When time allowed, she was supported in performing her own research. This eventually resulted in two first-author publications and the start of her own PhD project in 2011, on which this thesis is based.

## LIST OF PUBLICATIONS

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# Education Statement of the Graduate School <br> Experimental Plant Sciences 

| Issued to: | Joyce H.C. Woudenberg |
| :--- | :--- |
| Date: | 10 September 2015 |
| Group: | Evolutionary Phytopathology (CBS-KNAW) \& Phytopathology |
| University: | Wageningen University \& Research Centre |

- Writing a review or book chapter

MSc courses
Laboratory use of isotopes
5.5 credits*

## 2) Scientific Exposure

- EPS PhD student days

PhD day 2013, Leiden University
Nov 29, 2013
GET2GETHER event, StayOkay, Soest
Jan 29-30, 2015

## - EPS theme symposia

| EPS theme 2 symposium/WCS day: Interactions between plants and biotic agents, Utrecht University | Jan 24, 2013 |
| :--- | :--- | | EPS theme 2 symposium/WCS day: Interactions between plants and biotic agents, Utrecht University | Feb 20, 2015 |
| :--- | :--- |

- NWO Lunteren days and other National Platforms
- Seminars (series), workshops and symposia

CBS Seminar series (every Monday morning), Utrecht
Yearly meeting of KNVM Section Mycology, Utrecht
Mini-symposium 'Intraspecific pathogen variation-implications and opportunities', Wageningen
2011-2015
Nov 30, 2012

CBS Spring symposium 1F=?G, Amsterdam
Jan 22, 2013
CBS Spring symposium fungal genera and genomes, Amsterdam
Apr 10-11, 2013

Yearly meeting of KNVM Section Mycology, Utrecht
Apr 24-25, 2014
The second international workshop on Ascomycete systematics, Amsterdam
Nov 28, 2014

- Seminar plus
- International symposia and congresses

APS annual meeting 2012, Providence, RI, USA
Aug 04-08 2012
APS-MSA annual meeting 2013, Austin, TX, USA
Aug 10-14 2013
IMC 10, Bangkok, Thailand
Aug 03-08, 2014

- Presentations

APS annual meeting, Providence, RI, Poster: Multi-gene phylogeny reveals two new species-groups within Alternaria
CBS Seminar Series: Alternaria and allied genera
APS annual meeting, Austin, TX: Phylogenetic lineages within Alternaria and allied genera
CBS Seminar Series: Alternaria redefined
Friday morning meeting, Lab. of Phytopath., WU: A systematic revision of the genus Alternaria
CBS Spring symposium fungal genera and genomes: Alternaria redefined
IMC 10, Thailand, Poster: Large-spored Alternaria pathogens in section Porri disentangled
GET2GETHER event: Large-spored Alternaria pathogens in section Porri disentangled
Aug 06, 2012

IAB interview
Meeting with a member of the International Advisory Board of EPS
Dec 17, 2012
Aug 11, 2013
Dec 02, 2013
Feb 28, 2014
Apr 252014
Aug 03-08, 2014
Jan 29-30, 2015
Jan 05, 2015

- Excursions

Fungal Foray, APS annual meeting
Aug 04, 2012
22.0 credits*

| 3) In-Depth Studies | date |
| :---: | :---: |
| - EPS courses or other PhD courses |  |
| Introduction to Bioinformatics for Molecular Biologists | Nov 12-23, 2012 |
| The Power of RNA-seq | Dec 16-18, 2013 |
| - Journal club |  |
| - Individual research training |  |

Individual research training

## - Skill training courses

Techniques for writing and presenting a scientific paper
Information Literacy including endnote
Mini-symposium 'how to write a world-class paper'
Dec 11-14, 2012
Ot 17 2013
Crafting your career, CWTS/Rathenau institute
WGS PhD Workshop Carousel
Organisation of PhD students day, course or conference
Membership of Board, Committee or PhD council
Member of "evenementen commisie" CBS

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the
Educational Committee of EPS which comprises of a minimum total of 30 ECTS credits

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## Front and back cover:

Solanum lycopersicum leaf showing early blight symptoms (photograph by P.W. Crous) with composite picture of different Alternaria conidia arranged in a branching pattern on the front. Back cover insets: conidiophores and conidia of Alternaria arborescens, A. photistica and A. carthami (from top to bottom). Design: Marjan Vermaas.

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[^0]:    $\equiv$ Thyrospora radicina (Meier, Drechsler \& E.D. Eddy) Neerg., Bot. Tidsskr. 44: 361. 1939.
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    $\equiv$ Macrosporium smyrnii (P. Crouan \& H. Crouan) Sacc., Syll. Fungorum (Abellini) 4: 527. 1886.

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    ${ }^{4}$ EMSL analytical, Inc., 200 Route 130 North, Cinnaminson, NJ, USA

