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 26 sections. *Alternaria* sect. *Alternaria* contains most of the small-spored *Alternaria* species with concatenated conidia, including important plant, human and postharvest pathogens. Species within sect. *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 sect. *Alternaria* are supported by molecular data, whole-genome sequencing of nine *Alternaria* morphospecies supplemented with transcriptome sequencing of 12 *Alternaria* morphospecies as well as multi-gene sequencing of 168 *Alternaria* isolates was performed. The assembled genomes ranged in size from 33.3–35.2 Mb within sect. *Alternaria* and from 32.0–39.1 Mb for all *Alternaria* genomes. The number of repetitive sequences differed significantly between the different *Alternaria* genomes; ranging from 1.4–16.5 %. The repeat content within sect. *Alternaria* was relatively low with only 1.4–2.7 % of repeats. Whole-genome alignments revealed 96.7–98.2 % genome identity between sect. *Alternaria* isolates, compared to 85.1–89.3 % genome identity for isolates from other sections to the *A. alternata* reference genome. Similarly, 1.4–2.8 % and 0.8–1.8 % single nucleotide polymorphisms (SNPs) were observed in genomic and transcriptomic sequences, respectively, between isolates from sect. *Alternaria*, while the percentage of SNPs found in isolates from different sections compared to the *A. alternata* reference genome 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 sect. *Alternaria* (96.8 and 97.3 % conservation). Their potential discriminatory power within sect. *Alternaria* was tested next to nine commonly used gene regions in sect. *Alternaria*, namely the SSU, LSU, ITS, *gapdh*, *rpb2*, *tef1*, *Alt a 1*, *endoPG* and OPA10-2 gene regions. The phylogenies from the two gene regions with a relatively low conservation, KOG1058 and KOG1077, could not distinguish the described morphospecies within sect. *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* sect. *Alternaria* consists of only 11 phylogenetic species and one species complex. Thirty-five morphospecies, which cannot be distinguished based on the multi-gene phylogeny, are synonymised under *A. alternata*. By providing guidelines for the naming and identification of phylogenetic species in *Alternaria* sect. *Alternaria*, this manuscript provides a clear and stable species classification in this section.

Key words: *Alternaria alternata*, *Alternaria arborescens* species complex, Multi-gene phylogeny, Transcriptome sequencing, Whole-genome sequencing.

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INTRODUCTION

Alternaria sect. *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* (Woudenberg *et al.* 2013). *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 postharvest 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 sect. *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* species-groups, based on sporulation patterns, were made by Simmons (1995). More recent molecular-based studies revealed that *Alternaria* species cluster in several distinct species clades, now referred to as sections (Lawrence *et al.* 2013, Woudenberg *et al.* 2013), which do not always correlate with the species-groups that were delineated based on morphological characteristics. Currently, 26 *Alternaria* sections are recognised based on molecular phylogenies (Woudenberg *et al.* 2013, 2014, Grum-Grzhimaylo *et al.* 2015). So far, species within sect. *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 sect. *Alternaria* (Peever *et al.* 2004, Andrew *et al.* 2009). Multiple molecular methods have been tested or proposed for distinguishing 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 morphospecies described within sect. *Alternaria*.

The terms *forma specialis* and pathotype have been used to describe isolates that are morphologically indistinguishable from

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A. alternata, but infect particular hosts. At least 16 different *f. sp.* 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.

Because most morphospecies within sect. *Alternaria* cannot be distinguished based on sequences of 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 species (Lawrence *et al.* 2013). Since the introduction of next generation sequencing (NGS) many fungal genomes have become available for study, with the 1 000 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*, sect. *Brassicicola* (BioProject PRJNA34523), and *A. arborescens*, sect. *Alternaria* (BioProject PRJNA78243).

In this study, whole-genome sequences of four *Alternaria* spp. from sect. *Alternaria* and five *Alternaria* spp. from five other sections were generated, and supplemented by transcriptome sequences of nine *Alternaria* spp. from sect. *Alternaria* and three *Alternaria* spp. from three other sections of *Alternaria*. Species were selected based on their phylogenetic position (Woudenberg *et al.* 2013) in such a way that they are representative of the genus *Alternaria*, from the sister section of sect. *Alternaria*, sect. *Alternantherae* (*A. alternantherae*), to the most distant section, sect. *Crivellia* (*A. papaveraceae*). Within sect. *Alternaria*, species were selected based on their economic importance. Based on the genome and transcriptome data, two gene regions with 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 sect. *Alternaria*. Together with a standard multi-gene 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 18S nrDNA (SSU), the 28S 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), an attempt was made to create a clear and stable phylogenetic species classification in *Alternaria* sect. *Alternaria*.

MATERIAL AND METHODS

Isolates

One-hundred-and-sixty-eight *Alternaria* strains, including 64 (ex-) type or representative strains, present at the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands, were included in this study (Table 1) based on the phylogenetic position derived from their ITS sequence. A "representative isolate" refers to the strain 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.* 2009). Strains stored in liquid nitrogen were transferred to OA directly from the −185 °C storage.

DNA and RNA isolation for NGS

The genomes of four *Alternaria* spp. from sect. *Alternaria* and five *Alternaria* spp. from five other sections (Table 2) as well as the transcriptome profiles of nine *Alternaria* spp. from sect. *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 sect. *Alternaria*. Isolates were grown in malt peptone (MP) (Crous *et al.* 2009) supplemented with 1 × BME vitamin solution (Sigma-Aldrich® Chemie B.V., Zwijndrecht, The Netherlands) in a shaking incubator, at 25 °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 °C in a shaking incubator running at 700 rpm. Prewarmed QF buffer (50 °C) was used to elute the genomic DNA, and after precipitation the DNA was centrifuged at 4 °C for 20 min at 8 500 × 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 4 000 × g. When necessary, a final standard LiCl purification was performed.

NGS

DNA sequence and RNA sequence library preparation (500 bp insert) for Illumina® sequencing and the sequencing itself (100-bp paired end reads) were performed at the Applied Biosystematics Group of Plant Research International (PRI, Wageningen).

DNA sequence library preparation for Ion Torrent™ sequencing was performed at the CBS. The Ion Torrent™ library preparation was carried out using the Ion Xpress™ 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™ protocol using the Ion Xpress™ Plus Fragment

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]										
		SSU	LSU	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	OPA10-2	KOG1058	KOG1077
Alternaria alstroemeriae												
CBS 118808; E.G.S. 50.116^{R}	USA, *Alstroemeria* sp.	KP124917	KP124447	KP124296	KP124153	KP125071	KP124764	KP123845	KP123993	KP124601		
CBS 118809; E.G.S. 52.068; MAFF 1219^{T}	Australia, *Alstroemeria* sp.	KP124918	KP124448	KP124297	KP124154	KP125072	KP124765	np	KP123994	KP124602	KP125226	np
Alternaria alternantherae												
CBS 124392; HSAUP2798	China, *Solanum melongena*	**KC584506**	**KC584251**	**KC584179**	**KC584096**	**KC584633**	**KC584374**	KP123846	np	np	KP125227	KP125275
Alternaria alternata												
CBS 106.24; E.G.S. 38.029; ATCC 13963 (*A. mali*^{T})	USA, *Malus sylvestris*	KP124919	KP124449	KP124298	KP124155	KP125073	KP124766	KP123847	**AY295020**	**JQ800620**		
CBS 104.26	Unknown, unknown	KP124920	KP124450	KP124299	KP124156	KP125074	KP124767	KP123848	KP123995	KP124603		
CBS 107.27; ATCC 24463; QM 1736 (*A. citri*)	USA, *Citrus limonium*	KP124921	KP124451	KP124300	KP124157	KP125075	KP124768	KP123849	KP123996	KP124604		
CBS 154.31; IHEM 3320	USA, *Staphylea trifolia*	KP124922	KP124452	KP124301	KP124158	KP125076	KP124769	KP123851	KP123998	KP124606		
CBS 103.33; E.G.S. 35.182; IHEM 3319 (*A. soliaegyptiaca*^{T})	Egypt, soil	KP124923	KP124453	KP124302	KP124159	KP125077	KP124770	KP123852	KP123999	KP124607	KP125228	KP125276
CBS 106.34; E.G.S. 06.198; DSM 62019; MUCL 10030 (*A. lini*^{T})	Unknown, *Linum usitatissimum*	KP124924	KP124454	**Y17071**	**JQ646308**	KP125078	KP124771	KP123853	KP124000	KP124608		
CBS 117.44; E.G.S. 06.190; VKM F-1870 (*A. godetiae*^{T})	Denmark, *Godetia* sp.	KP124925	KP124455	KP124303	KP124160	KP125079	KP124772	KP123854	KP124001	KP124609	KP125229	KP125277
CBS 102.47; E.G.S. 02.062 (*A. citri*^{R})	USA, *Citrus sinensis*	KP124926	KP124456	KP124304	KP124161	KP125080	KP124773	KP123855	KP124002	KP124610		
CBS 174.52; E.G.S. 39.1613; IMI 068086; QM 1278	USA, *Anemone occidentalis*	**KC584578**	**DQ678068**	**KC584228**	**KC584152**	**KC584704**	**DQ677964**	KP123856	KP124003	KP124611		
CBS 175.52; E.G.S. 35.1619; IMI 068085; QM 1277	USA, *Juncus mertensianus*	**KC584577**	**KC584320**	**KC584227**	**KC584151**	**KC584703**	**KC584445**	KP123857	KP124004	KP124612		
CBS 107.53; DSM 3187; IFO 5778 (*A. kikuchiana*)	Japan, *Pyrus pyrifolia*	KP124927	KP124457	KP124305	KP124162	KP125081	KP124774	KP123858	KP124005	KP124613		
CBS 686.68; LCP 1988 (*A. tenuissima*)	Sahara, desert sand	KP124928	KP124458	KP124306	KP124163	KP125082	KP124775	KP123859	KP124006	KP124614		
CBS 826.68; IMI 265857 (*A. nobilis*)	Germany, *Lolium* sp.	KP124929	KP124459	KP124307	KP124164	KP125083	KP124776	KP123860	KP124007	np		
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 (*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*^{T})	USA, *Quercus* sp.	KP124938	KP124468	KP124316	KP124172	KP125092	KP124784	KP123869	KP124016	KP124623	KP125230	KP125278
CBS 195.86; E.G.S. 36.172; DAOM 185214 (*A. angustiovoidea*^{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		

(*continued on next page*)

Table 1. (Continued).

Species name and strain 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 479.90; E.G.S. 29.028 (*A. pellucida*^{T})	Japan, *Citrus unshiu*	KP124941	KP124471	KP124319	KP124174	KP125095	KP124787	KP123870	KP124019	KP124626	KP125232	KP125280
CBS 595.93 (*A. rhadina*^{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		
CBS 916.96; E.G.S. 34.016; CBS 110977; CBS 115616; IMI 254138^{T}	India, *Arachis hypogaea*	**KC584507**	**DQ678082**	**AF347031**	**AY278808**	**KC584634**	**KC584375**	**AY563301**	**JQ811978**	KP124632	KP125233	KP125281
CBS 918.96; E.G.S. 34.015; IMI 255532 (*A. tenuissima*^{R})	UK, *Dianthus chinensis*	**KC584567**	**KC584311**	**AF347032**	**AY278809**	**KC584693**	**KC584435**	**AY563302**	KP124026	KP124633	KP125234	KP125282
CBS 911.97; IMI 056271 (*A. tenuissima*)	India, *Artemisia brevifolia*	KP124948	KP124478	KP124326	KP124181	KP125102	KP124794	KP123875	KP124027	KP124634		
CBS 639.97; IMI 366417	Greece, *Helianthus annuus*	KP124949	KP124479	KP124327	KP124182	KP125103	KP124795	KP123876	KP124028	KP124635		
CBS 102595; E.G.S. 45.100 (*A. limoniasperae*^{T})	USA, *Citrus jambhiri*	**KC584540**	**KC584284**	**FJ266476**	**AY562411**	**KC584666**	**KC584408**	**AY563306**	KP124029	KP124636	KP125235	KP125283
CBS 102596; E.G.S. 45.090 (*A. citrimacularis*^{T})	USA, *Citrus jambhiri*	KP124950	KP124480	KP124328	KP124183	KP125104	KP124796	KP123877	KP124030	KP124637	KP125236	KP125284
CBS 102598; E.G.S. 46.141 (*A. citriarbusti*^{T})	USA, *Minneola tangelo*	KP124951	KP124481	KP124329	KP124184	KP125105	KP124797	KP123878	KP124031	KP124638	KP125237	KP125285
CBS 102599; E.G.S. 44.166 (*A. turkisafria*^{T})	Turkey, *Minneola tangelo*	KP124952	KP124482	KP124330	KP124185	KP125106	KP124798	KP123879	KP124032	KP124639	KP125238	KP125286
CBS 102600; E.G.S. 39.181; ATCC 38963 (*A. toxicogenica*^{T})	USA, *Citrus reticulata*	KP124953	KP124483	KP124331	KP124186	KP125107	KP124799	KP123880	KP124033	KP124640	KP125239	KP125287
CBS 102602; E.G.S. 44.160 (*A. perangusta*^{T})	Turkey, *Minneola tangelo*	KP124954	KP124484	KP124332	KP124187	KP125108	KP124800	KP123881	**AY295023**	KP124641	KP125240	KP125288
CBS 102603; E.G.S. 45.011 (*A. interrupta*^{T})	Israel, *Minneola tangelo*	KP124955	KP124485	KP124333	KP124188	KP125109	KP124801	KP123882	KP124034	KP124642		
CBS 102604; E.G.S. 45.007 (*A. dumosa*^{T})	Israel, *Minneola tangelo*	KP124956	KP124486	KP124334	**AY562410**	KP125110	KP124802	**AY563305**	KP124035	KP124643	KP125241	KP125289
CBS 109455	Canada, human arm tissue	KP124957	KP124487	KP124335	KP124189	KP125111	KP124803	KP123883	KP124036	KP124644		
CBS 109803	Germany, human skin	KP124958	KP124488	KP124336	KP124190	KP125112	KP124804	KP123884	KP124037	KP124645		
CBS 110027	Germany, human eye	KP124959	KP124489	KP124337	KP124191	KP125113	KP124805	KP123885	KP124038	KP124646		
CBS 110977; E.G.S. 34.016; CBS 916.96; CBS 115616^{T}	India, *Arachis hypogaea*	**KC584507**	**DQ678082**	**AF347031**	**AY278808**	**KC584634**	**KC584375**	**AY563301**	**JQ811978**	KP124647		
CBS 112249	Unknown, unknown	KP124960	KP124490	KP124338	KP124192	KP125114	KP124806	KP123886	KP124039	KP124648		
CBS 112251 (*A. arborescens*)	Unknown, unknown	KP124961	KP124491	KP124339	KP124193	KP125115	KP124807	KP123887	KP124040	KP124649		
CBS 112252 (*A. tenuissima*)	Unknown, unknown	KP124962	KP124492	KP124340	KP124194	KP125116	KP124808	KP123888	KP124041	KP124650		
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		

Table 1. (Continued).

Species name and strain 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 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^{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 (*A. brassicinae*^{T})	USA, *Brassica oleracea*	KP124978	KP124508	KP124356	KP124210	KP125132	KP124824	KP123904	KP124057	KP124667	KP125242	KP125290
CBS 118812; E.G.S. 37.050 (*A. daucifolii*^{T})	USA, *Daucus carota*	**KC584525**	**KC584269**	**KC584193**	**KC584112**	**KC584652**	**KC584393**	KP123905	KP124058	KP124668	KP125243	KP125291
CBS 118814; E.G.S. 44.048 (*A. tomaticola*^{T})	USA, *Solanum lycopersicum*	KP124979	KP124509	KP124357	KP124211	KP125133	KP124825	KP123906	KP124059	KP124669	KP125244	KP125292
CBS 118815; E.G.S. 51.132 (*A. tomaticola*^{R})	USA, *Solanum lycopersicum*	KP124980	KP124510	KP124358	KP124212	KP125134	KP124826	KP123907	KP124060	KP124670		
CBS 118818; E.G.S. 31.032 (*A. vaccinii*^{T})	USA, *Vaccinium* sp.	KP124981	KP124511	KP124359	KP124213	KP125135	KP124827	KP123908	KP124061	KP124671	KP125245	KP125293
CBS 119115	Greece, *Prunus* sp.	KP124982	KP124512	KP124360	KP124214	KP125136	KP124828	KP123909	KP124062	np		
CBS 119399; E.G.S. 39.189 (*A. postmessia*^{T})	USA, *Minneola tangelo*	KP124983	KP124513	KP124361	**JQ646328**	KP125137	KP124829	KP123910	KP124063	KP124672	KP125246	KP125294
CBS 119408; E.G.S. 40.140 (*A. herbiphorbicola*^{T})	USA, *Euphorbia esula*	KP124984	KP124514	KP124362	**JQ646326**	KP125138	KP124830	**JQ646410**	KP124064	KP124673	KP125247	KP125295
CBS 119543; E.G.S. 12.160 (*A. citricancri*^{T})	USA, *Citrus paradisi*	KP124985	KP124515	KP124363	KP124215	KP125139	KP124831	KP123911	KP124065	KP124674	KP125248	KP125296
CBS 120829	Greece, *Punica granatum*	KP124986	KP124516	KP124364	KP124216	KP125140	KP124832	KP123912	KP124066	KP124675		
CBS 121336; E.G.S. 37.005; ATCC 11680 (*A. palandui*^{T})	USA, *Allium* sp.	KP124987	KP124517	**KJ862254**	**KJ862255**	KP125141	KP124833	**KJ862259**	KP124067	KP124676	KP125249	KP125297
CBS 121344; E.G.S. 45.003 (*A. turkisafria*^{R})	Israel, *Minneola tangelo*	KP124988	KP124518	KP124365	KP124217	KP125142	KP124834	KP123913	KP124068	KP124677		
CBS 121346; E.G.S. 45.056 (*A. turkisafria*^{R})	South Africa, *Minneola tangelo*	KP124989	KP124519	KP124366	KP124218	KP125143	KP124835	KP123914	KP124069	KP124678		
CBS 121348; E.G.S. 50.070 (*A. platycodonis*^{T})	China, *Platycodon grandiflorus*	KP124990	KP124520	KP124367	KP124219	KP125144	KP124836	KP123915	KP124070	KP124679	KP125250	KP125298
CBS 121454; E.G.S. 46.069 (*A. destruens*^{T})	USA, *Cuscuta gronovii*	KP124991	KP124521	**AF278836**	**AY278812**	KP125145	KP124837	**JQ646402**	KP124071	KP124680	KP125251	KP125299
CBS 121455; E.G.S. 50.078 (*A. broussonetiae*^{T})	China, *Broussonetia papyrifera*	KP124992	KP124522	KP124368	KP124220	KP125146	KP124838	KP123916	KP124072	KP124681	KP125252	KP125300
CBS 121456; E.G.S. 50.080; HSAUP 9600197 (*A. sanguisorbae*^{T})	China, *Sanguisorba officinalis*	KP124993	KP124523	KP124369	KP124221	KP125147	KP124839	KP123917	KP124073	KP124682	KP125253	KP125301
CBS 121492; HSAUP0207 (*Ulocladium cucumisis*)	China, *Cucumis melo*	KP124994	KP124524	KP124370	KP124222	KP125148	KP124840	KP123918	KP124074	KP124683		

(*continued on next page*)

Table 1. (Continued).

Species name and strain 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 121544; E.G.S. 38.022 (*A. caudata*^{R})	USA, *Cucumis sativus*	KP124995	KP124525	KP124371	KP124223	KP125149	KP124841	KP123919	KP124075	KP124684		
CBS 121547; E.G.S. 50.048 (*A. yali-inficiens*^{T})	China, *Pyrus bretschneideri*	KP124996	KP124526	KP124372	KP124224	KP125150	KP124842	KP123920	KP124076	KP124685		
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 (*A. seleniiphila*^{T})	USA, *Stanleya pinnata*	KP125005	KP124535	KP124381	KP124233	KP125159	KP124851	KP123929	KP124085	KP124694		
CBS 127672; E.G.S. 52.122 (*A. astragali*^{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; QM1765 (*A. geophila*^{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; 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		
CBS 105.49 (*A. alternata*)	Italy, contaminant blood culture	KP125020	KP124550	KP124396	KP124248	KP125174	KP124866	KP123944	KP124100	KP124709		
CBS 126.60; IMI 081622 (*A. maritima*)	UK, wood	**GU456294**	**GU456317**	KP124397	KP124249	KP125175	KP124867	**JQ646390**	KP124101	KP124710		
CBS 750.68; LCP 68.1989 (*A. tenuissima*)	France, *Phaseolus vulgaris*	KP125021	KP124551	KP124398	KP124250	KP125176	KP124868	KP123945	KP124102	KP124711		
CBS 102605; E.G.S. 39.128 (*A. arborescens*^{T})	USA, *Solanum lycopersicum*	**KC584509**	**KC584253**	**AF347033**	**AY278810**	**KC584636**	**KC584377**	**AY563303**	**AY295028**	KP124712	KP125255	KP125303
CBS 109730 (*A. arborescens*)	USA, *Solanum lycopersicum*	KP125022	KP124552	KP124399	KP124251	KP125177	KP124869	KP123946	KP124103	KP124713		
CBS 112633; CPC 4244 (*A. arborescens*)	South Africa, *Malus domestica*	KP125023	KP124553	KP124400	KP124252	KP125178	KP124870	KP123947	KP124104	KP124714		

Table 1. (Continued).

Species name and strain 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 112749; CPC 4245 (*A. arborescens*)	South Africa, *Malus domestica*	KP125024	KP124554	KP124401	KP124253	KP125179	KP124871	KP123948	KP124105	KP124715		
CBS 115189; CPC 4345 (*A. arborescens*)	South Africa, *Citrus clementina*	KP125025	KP124555	KP124402	KP124254	KP125180	KP124872	KP123949	KP124106	KP124716		
CBS 115516; CPC 4247 (*A. arborescens*)	South Africa, *Malus domestica*	KP125026	KP124556	KP124403	KP124255	KP125181	KP124873	KP123950	KP124107	KP124717		
CBS 115517; CPC 4246 (*A. arborescens*)	South Africa, *Malus domestica*	KP125027	KP124557	KP124404	KP124256	KP125182	KP124874	KP123951	KP124108	KP124718		
CBS 116329 (*A. alternata*)	Germany, *Malus domestica*	KP125028	KP124558	KP124405	KP124257	KP125183	KP124875	KP123952	KP124109	KP124719		
CBS 117587 (*A. alternata*)	Netherlands, *Brassica* sp.	KP125029	KP124559	KP124406	KP124258	KP125184	KP124876	KP123953	KP124110	KP124720		
CBS 118389; E.G.S. 90.131 (*A. gaisen*^{R})	Japan, *Pyrus pyrifolia*	KP125030	KP124560	KP124407	KP124259	KP125185	KP124877	KP123954	KP124111	KP124721		
CBS 119544; E.G.S. 43.072 (*A. cerealis*^{T})	New Zealand, *Avena sativa*	KP125031	KP124561	KP124408	**JQ646321**	KP125186	KP124878	KP123955	KP124112	KP124722	KP125256	KP125304
CBS 119545; E.G.S. 48.130 (*A. senecionicola*^{T})	New Zealand, *Senecio skirrhodon*	KP125032	KP124562	KP124409	KP124260	KP125187	KP124879	KP123956	KP124113	KP124723	KP125257	KP125305
CBS 123235 (*A. alternata*)	Denmark, human toenail	KP125033	KP124563	KP124410	KP124261	KP125188	KP124880	KP123957	KP124114	KP124724		
CBS 123266 (*A. alternata*)	Denmark, human toenail	KP125034	KP124564	KP124411	KP124262	KP125189	KP124881	KP123958	KP124115	KP124725		
CBS 123267 (*A. alternata*)	Denmark, human nail	KP125035	KP124565	KP124412	KP124263	KP125190	KP124882	KP123959	KP124116	KP124726		
CBS 124274 (*A. arborescens*)	Denmark, *Prunus* sp.	KP125036	KP124566	KP124413	KP124264	KP125191	np	KP123960	KP124117	KP124727		
CBS 124281 (*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^{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^{T}	India, *Cuminum cyminum*	KP125043	KP124573	KP124420	**JQ646305**	KP125198	KP124889	KP123967	KP124124	KP124734	KP125259	np
CBS 110.50; MUCL 10012 (*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. 43.145; IMI 368045 (*A. rhizophorae*^{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*^{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^{T}	India, *Eichhornia crassipes*	KP125049	KP124579	**KC146356**	KP124276	KP125204	KP124895	KP123973	KP124130	KP124740	KP125262	KP125309
CBS 119778; E.G.S. 45.026; IMI 37968^{R}	Indonesia, *Eichhornia crassipes*	KP125050	KP124580	KP124426	KP124277	KP125205	KP124896	np	KP124131	KP124741	KP125263	KP125310
Alternaria gaisen												

(*continued on next page*)

Table 1. (Continued).

Species name and strain 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 632.93; E.G.S. 90.512^{R}	Japan, *Pyrus pyrifolia*	**KC584531**	**KC584275**	**KC584197**	**KC584116**	**KC584658**	**KC584399**	KP123974	**AY295033**	KP124742	KP125264	KP125311
CBS 118488; E.G.S. 90.391^{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^{T}	Zimbabwe, *Gossypium* sp.	KP125054	KP124584	KP124430	**JQ646312**	KP125209	KP124900	**JQ646395**	KP124135	KP124746		
CBS 107.36 (*A. grisea*^{T})	Indonesia, soil	KP125055	KP124585	KP124431	**JQ646310**	KP125210	KP124901	**JQ646393**	KP124136	KP124747		
CBS 102597; E.G.S. 45.114 (*A. tangelonis*^{T})	USA, *Minneola tangelo*	KP125056	KP124586	KP124432	KP124281	KP125211	KP124902	KP123978	KP124137	KP124748	KP125266	KP125313
CBS 102601; E.G.S. 45.017 (*A. colombiana*^{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 354A^{R}	New Zealand, *Iris* sp.	KP125058	KP124588	KP124434	KP124283	KP125213	KP124904	KP123980	KP124139	KP124750	KP125268	np
CBS 118486; E.G.S. 43.014^{T}	Australia, *Iris* sp.	KP125059	KP124589	KP124435	KP124284	KP125214	KP124905	KP123981	KP124140	KP124751		
CBS 118487; E.G.S. 44.147^{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^{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^{R}	USA, *Nicotiana tabacum*	KP125067	KP124597	KP124443	KP124292	KP125227	KP124913	KP123989	KP124149	KP124760		
CBS 121333; E.G.S. 30.051^{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
CBS 114.35	Unknown, *Solanum lycopersicum*	KP125070	KP124600	KP124446	KP124295	KP125225	KP124916	KP123992	KP124152	KP124763	KP125274	KP125317

[1] 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(s)	Section	Sequencing method	Size (Mb)	Coverage (approx.)	% Repeats	% Identity	% SNPs[2]
A. alternata	CBS 916.96[3]	*Alternaria*	Illumina	33.3	40×	1.4	na[3]	na[3]
A. arborescens[1]	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×	1.7	98.1	1.4
A. gaisen	CBS 118488	*Alternaria*	Illumina	35.2	182×	1.8	96.7	2.8
A. tenuissima (now *A. alternata*)	CBS 918.96	*Alternaria*	Illumina	33.5	260×	1.4	98.2	1.5
A. alternantherae	CBS 124392	*Alternantherae*	Illumina	35.0	210×	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×	11.9	87.2	9.5
A. infectoria	CBS 210.86	*Infectoriae*	Illumina	36.5	200×	5.3	85.1	10.3
A. papaveraceae	CBS 116607	*Crivellia*	Illumina	33.8	220×	5.3	85.8	10.3
A. brassicicola[1]	ATCC 96836 = CBS 118699	*Brassicicola*	–	32.0	–	7.1	86.6	–

[1] Publicly available genomes; *A. arborescens* downloaded from NCBI, *A. brassisicola* downloaded from JGI (http://genome.jgi-psf.org/Altbr1/Altbr1.home.html).
[2] SNPs / covered base (>10×), duplicates removed.
[3] Reference isolate.

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×), duplicates removed.

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™ Template OT2 200 Kit (Thermo Fisher Scientific) and associated protocol. The enrichment percentage was determent via the Ion Sphere™ Quality Control Kit (Thermo Fisher Scientific) and was performed between the emulsion PCR and the enrichment step. Sequencing was performed using the Ion PGM™ Sequencing 200 Kit v. 2 (Thermo Fisher Scientific) with an Ion 318™ Chip Kit v. 2 (Thermo Fisher Scientific).

Genome assembly and mapping

De novo genome assembly of the Illumina® 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™ 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) 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 (Qual = 50), depth (DP = 10) and allelic frequency (AF = 0.9).

Conserved eukaryotic orthologous group (KOG) proteins were identified using the Core Eukaryotic Genes Mapping Approach (CEGMA) pipeline (Parra *et al.* 2007). The conservation table was constructed from the five available genomes of sect. *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 1 000 bootstrap replicates.

PCR and sequencing

DNA extraction for gene sequencing was performed using the UltraClean™ 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 Woudenberg *et al.* (2013) and the *Alt a 1* gene as described in Woudenberg *et al.* (2014). 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′-GAG TCA CGT TAY CGC ASC-3′) and KOG1058R2 (5′-TGG CTK ACG GAR ACG-3′) and KOG1077F2 (5′-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® 2720 Thermal Cycler (Thermo Fisher Scientific), in a total volume of 12.5 µL. The PCR mixtures consisted of 1 µL genomic DNA, 1× NH_4 reaction buffer (Bioline, Luckenwalde, Germany), 2 mM (*endoPG*, OPA10-2) or 1.6 mM $MgCl_2$ (KOG1058, KOG1077), 20 µM of each dNTP, 0.2 µM 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 °C followed by 40 cycles of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C for *endoPG*, 35 cycles of 30 s at 94 °C, 30 s at 62 °C and 45 s at 72 °C for OPA10-2, and 35 cycles of 30 s at 94 °C, 30 s at 59 °C and 60 s at 72 °C for KOG1058 and KOG1077, and a final elongation step of 7 min at 72 °C. The PCR products were sequenced in both directions using the PCR primers and a BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific), and analysed with an ABI Prism 3730xl DNA Analyser (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 OPA10-2 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).

Phylogenetic species recognition and naming in *Alternaria* sect. *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 sect. *Alternaria*, are described with the species below and listed in a table which can be downloaded from the CBS-KNAW website (www.cbs.knaw.nl/index.php/studies-in-mycology) or requested from the author. 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 sect. *Alternaria*.

To further standardise the taxonomic terms used, the trinomial system introduced by Rotem (1994) is favoured. 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 should 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 to the Discussion.

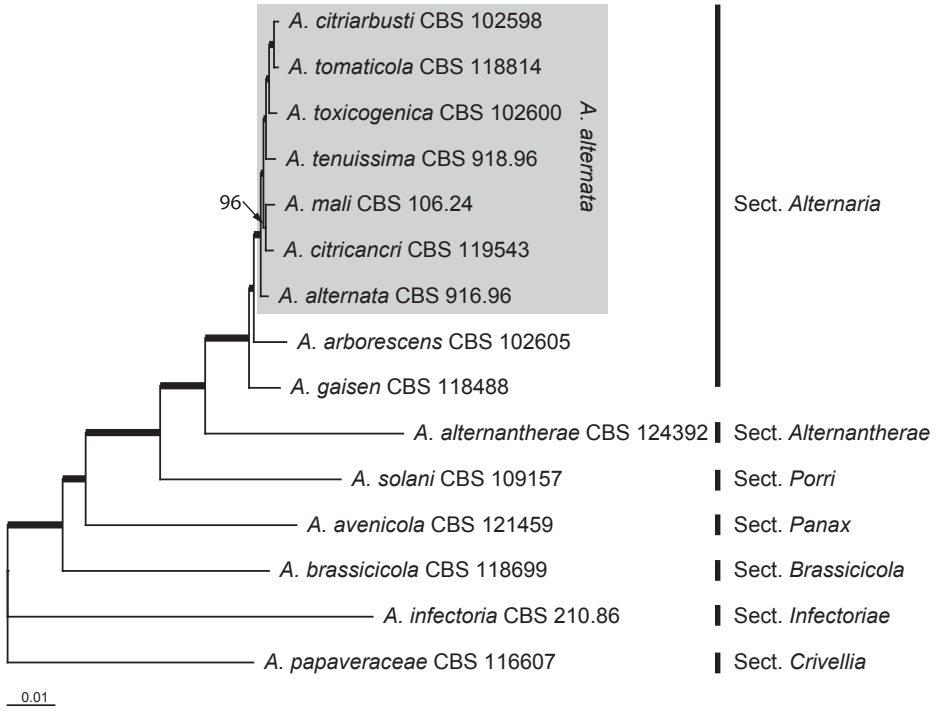


Fig. 1. PhyML tree based on the whole-genome and transcriptome reads of 15 *Alternaria* species using REALPHY. The bootstrap support values are given at the nodes; thickened lines indicate a fully supported node. The grey box represents species which are now synonymised under *A. alternata*. The tree was rooted to *A. papaveraceae* (CBS 116607).

RESULTS

NGS

Nine *Alternaria* (morpho)species were sequenced using Ion Torrent™ or Illumina® sequencing technologies, yielding between 38× and >260× average genome coverage (Table 2). The assembled genomes ranged in size from 33.3–35.2 Mb within sect. *Alternaria* and from 32.0–39.1 Mb for all *Alternaria* genomes (Table 2). To characterise the assembled genomes, the repetitive complement of each individual genome was identified and classified using a combination of *de novo* prediction and identification of known repetitive elements. Surprisingly, the number of repetitive sequences differed significantly between different *Alternaria* genomes. Within sect. *Alternaria*, the number of repetitive sequences is relatively 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, whole-genome alignments to the reference genome of *A. alternata* (CBS 916.96) were performed. These alignments revealed 96.7–98.2 % genome identity within sect. *Alternaria* compared to 85.1–89.3 % genome identity between isolates from other sections with *A. alternata*. Furthermore, the number of single nucleotide polymorphisms (SNPs) between the different species were assessed by mapping genomic reads to the reference genome of *A. alternata* (CBS 916.96). Between isolates from sect. *Alternaria*, 1.4–2.8 % SNPs were observed, 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, deep transcriptome sequences of 12 isolates were derived that were mapped to the reference isolate of *A. alternata* (CBS 916.96). In this case, 0.8–1.8 % SNPs among the isolates from sect. *Alternaria* were observed, while the isolates from other sections displayed 6.1–8.5 % SNPs (Table 3).

Marker genes with potential discriminatory power were identified by predicting a set of conserved eukaryotic genes (KOG) in the genomes of the five assembled sect. *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. Primers spanning the first 5 introns of KOG1058 and KOG1077 were designed (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 1 750 944 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 Woudenberg *et al.* 2013). Section *Alternantherae* and sect. *Porri* are the sister sections of sect. *Alternaria*, while sect. *Infectoriae* and sect. *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

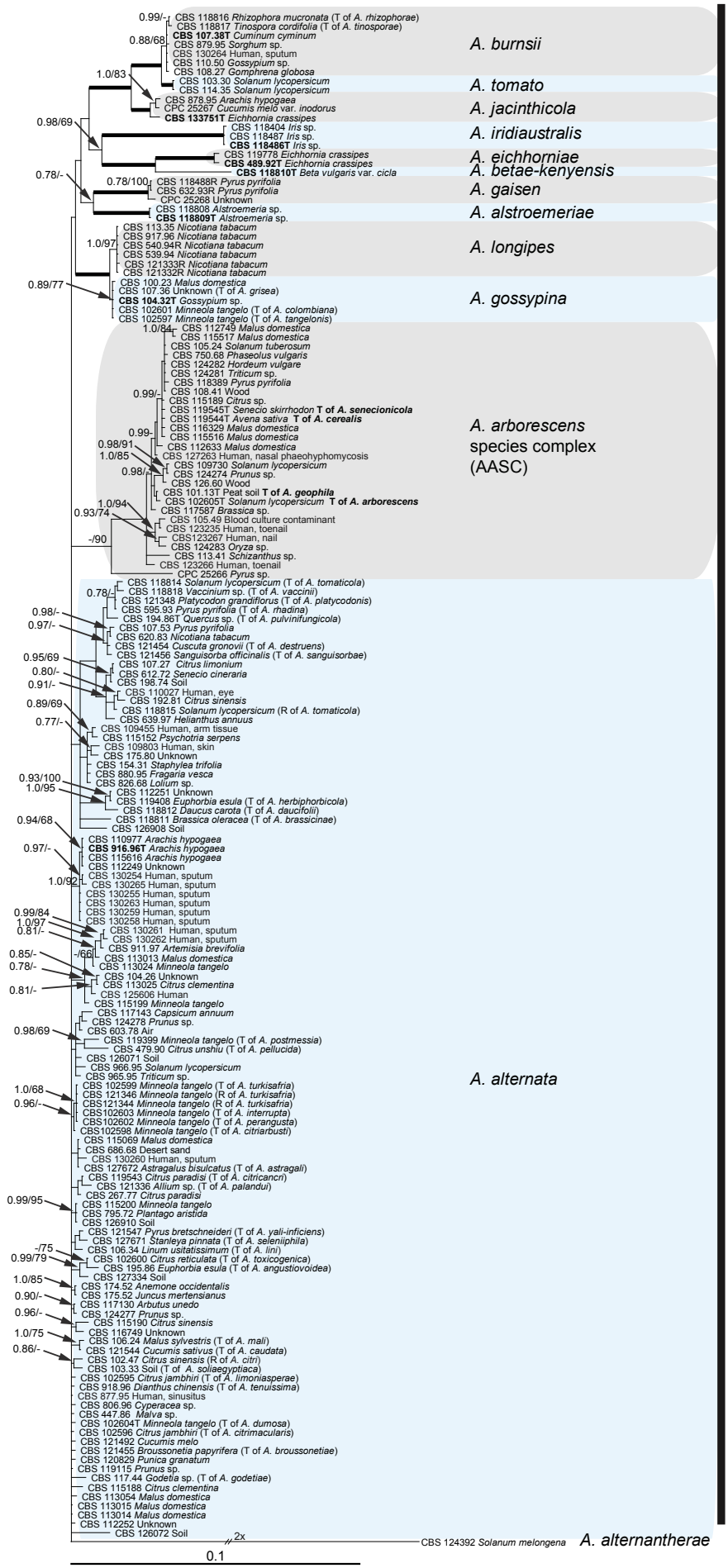
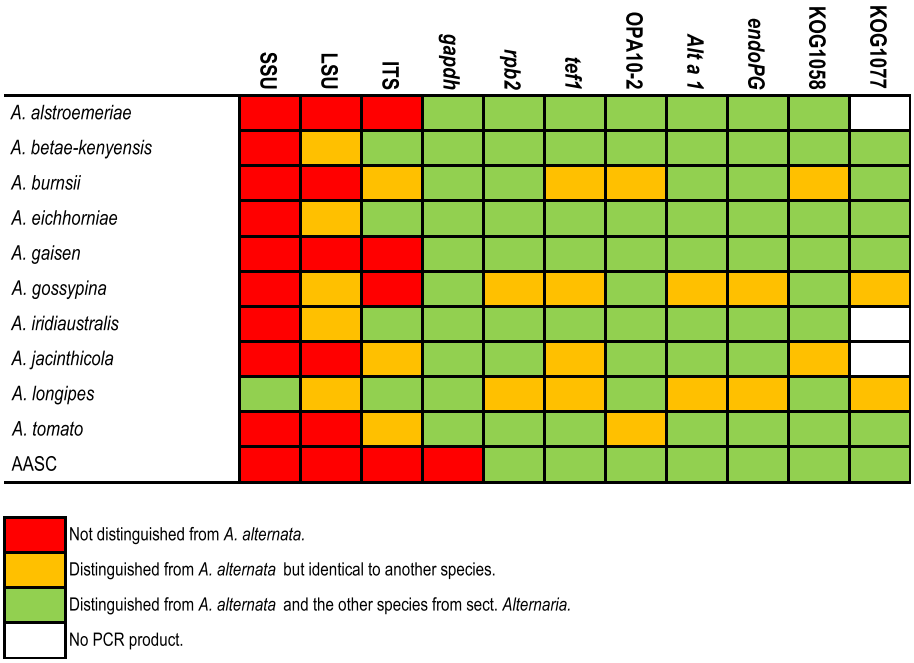


Fig. 2. Bayesian 50 % majority rule consensus tree based on the ITS, *gapdh*, *tef1*, *rpb2*, *Alt a 1*, *endoPG* and OPA10-2 sequences of 168 *Alternaria* strains. The Bayesian posterior probabilities >0.75 (PP) and RAxML bootstrap support values >65 (ML) are given at the nodes (PP / ML). Thickened 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. 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 sect. *Alternaria*.



included as missing data in the combined analysis. The aligned sequences of the SSU (1 021 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 1 021 / 849 aligned characters) these genes were excluded from the multi-gene phylogeny. The multi-gene phylogeny based on the remaining seven gene regions contained 3 651 characters including alignment gaps, which, after discarding the burn-in phase, resulted in a 50 % majority rule consensus tree based on 15 002 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 4). Since the KOG1077 sequences could not separate *A. longipes* from *A. gossypina*, no further effort was put 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 are synonymised 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 phylogenetic species recognised here on its own (Table 4). The commonly used *gapdh* sequence could distinguish all species, except the *A. arborescens* species complex (AASC), from *A. alternata*. Five genes, namely *rpb2*, OPA10-2, *Alt a 1*, *endoPG* and KOG1058, could separate all species from *A. alternata*, but failed 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, and are summarised in a table which can be downloaded from the CBS-KNAW website (www.cbs.knaw.nl/index.php/studies-in-mycology) or requested from the author.

Phylogenetic species in sect. *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.

≡ *Macrosporium tenuissimum* (Nees & T. Nees) Fr., Syst. Mycol. 3: 374. 1832. (nom. sanct.)

≡ *Clasterosporium tenuissimum* (Nees & T. Nees: Fr.) Sacc., Sylloge Fungorum (Abellini) 4: 393. 1886.

≡ *Alternaria tenuissima* (Nees & T. Nees: Fr.) Wiltshire, Trans. Brit. Mycol. Soc. 18: 157. 1933.

= *Macrosporium fasciculatum* Cooke & Ellis, Grevillea 6: 6. 1877.

≡ *Alternaria fasciculata* (Cooke & Ellis) I.R. Jones & Grout, Bull. Torrey Bot. Club 24: 257. 1897.

= *Macrosporium caudatum* Cooke & Ellis, Grevillea 6: 87. 1878.

≡ *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.

= *Macrosporium erumpens* Cooke, Grevillea 12: 32. 1883.

≡ *Alternaria erumpens* (Cooke) Joly, Le Genre *Alternaria*: 199. 1964.

= *Macrosporium martindalei* Ellis & G. Martin, Amer. Naturalist 18: 189. 1884.

≡ *Alternaria martindalei* (Ellis & G. Martin) Joly, Le Genre *Alternaria*: 209. 1964.

= *Macrosporium polytrichi* Peck, Rep. (Annual) NewYork State Mus. Nat. Hist. 34: 31. 1890.

= *Macrosporium podophylli* Ellis & Everh., Proc. Acad. Nat. Sci. Philadelphia 43: 92. 1891.

≡ *Alternaria podophylli* (Ellis & Everhart) Joly, Le Genre *Alternaria*: 212. 1964.

= *Macrosporium seguierii* Allescher, Hedwigia 33: 75. 1894.

= *Macrosporium amaranthi* Peck, Bull. Torrey Bot. Club 22: 493. 1895.

≡ *Alternaria amaranthi* (Peck) J. van Hook, Proc. Indiana Acad. Sci. 1920: 214. 1921.

= *Alternaria citri* Ellis & N. Pierce, Bot. Gaz. (Crawfordville) 33: 234. 1902.

= *Alternaria ribis* Bubák & Ranojević, Ann. Mycol. 8: 400. 1910.

= *Alternaria mali* Roberts, J. Agric. Res. 2: 58. 1914.

= *Alternaria palandui* Ayyangar, Bull. Agric. Res. Inst., Pusa 179: 14. 1928.

= *Alternaria lini* Dey, Indian J. Agric. Sci. 3: 881. 1933.

= *Alternaria tenuissima* var. *godetiae* Neerg., Trans. Brit. Mycol. Soc. 18: 157. 1933.

≡ *Alternaria godetiae* (Neerg.) Neerg., Aarsberetn. J. E. Ohlens Enkes Plantepatol. Lab. 10: 14. 1945.

= *Macrosporium pruni-mahalebi* Săvulescu & Sandu, Hedwigia 75: 228. 1935.

= *Alternaria rumicicola* R.L. Mathur, J.P. Agnihotri & Tyagi, Curr. Sci. 31: 297. 1962.

= *Alternaria tenuissima* var. *verruculosa* S. Chowdhury, Proc. Natl. Acad. Sci. India, Sect. B, Biol. Sci. 36: 301. 1966.

= *Alternaria angustiovoidea* E.G. Simmons, Mycotaxon 25: 198. 1986.

= *Alternaria pellucida* E.G. Simmons, Mycotaxon 37: 102. 1990.

= *Alternaria rhadina* E.G. Simmons, Mycotaxon 48: 101. 1993.

= *Alternaria destruens* E.G. Simmons, Mycotaxon 68: 419. 1998.

= *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.

= *Alternaria tenuissima* var. *alliicola* T.Y. Zhang, Mycotaxon 72: 450. 1999.

= *Alternaria toxicogenica* E.G. Simmons, Mycotaxon 70: 294. 1999.

= *Alternaria turkisafria* E.G. Simmons, Mycotaxon 70: 290. 1999.

= *Alternaria sanguisorbae* M.X. Gao & T.Y. Zhang, Mycosystema 19: 456. 2000.

= *Alternaria platycodonis* Z.Y. Zhang & H. Zhang, Flora Fungorum Sin., *Alternaria*: 66. 2003.

= *Alternaria yali-inficiens* R.G. Roberts [as '*yaliinficiens*'], Pl. Dis. 89: 142. 2005.

= *Alternaria astragali* Wangeline & E.G. Simmons, Mycotaxon 99: 84. 2007.

= *Alternaria brassicinae* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 532. 2007.

= *Alternaria citricancri* E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 542. 2007.

= *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.

= *Alternaria seleniiphila* Wangeline & E.G. Simmons, Mycotaxon 99: 86. 2007.

= *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, the well-established name of the type species of *Alternaria*, *A. alternata* is retained 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 *A. malvae* is not synonymised 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. Therefore *A. lini* is synonymised 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, no unique fixed nucleotides are assigned to *A. alternata*. Three *formae speciales* of *A. alternata* are currently recognised; *A. alternata f. sp. mali* for isolates producing the AM-toxin, *f. sp. fragariae* for isolates producing the AF-toxin, and *f. sp. citri* with two pathotypes, *i.e. f. sp. citri* pathotype rough lemon for isolates producing the ACR-toxin, and *f. sp. citri* pathotype tangerine for isolates producing the ACT-toxin.

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 (T), 473 (A); ***endoPG*** position 10 (T), 286 (T), 295 (T), 372 (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 easily be distinguished from *A. alternata* using molecular data. 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 (G), 162 (G), 168 (T), 509 (A); ***rpb2*** position 6 (T), 549 (G); ***tef1*** position 12 (C), 31 (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 (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. 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 µm.

= *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 easily be distinguished from *A. alternata* using molecular data. 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 *A. grossulariae* is not synonymised 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).

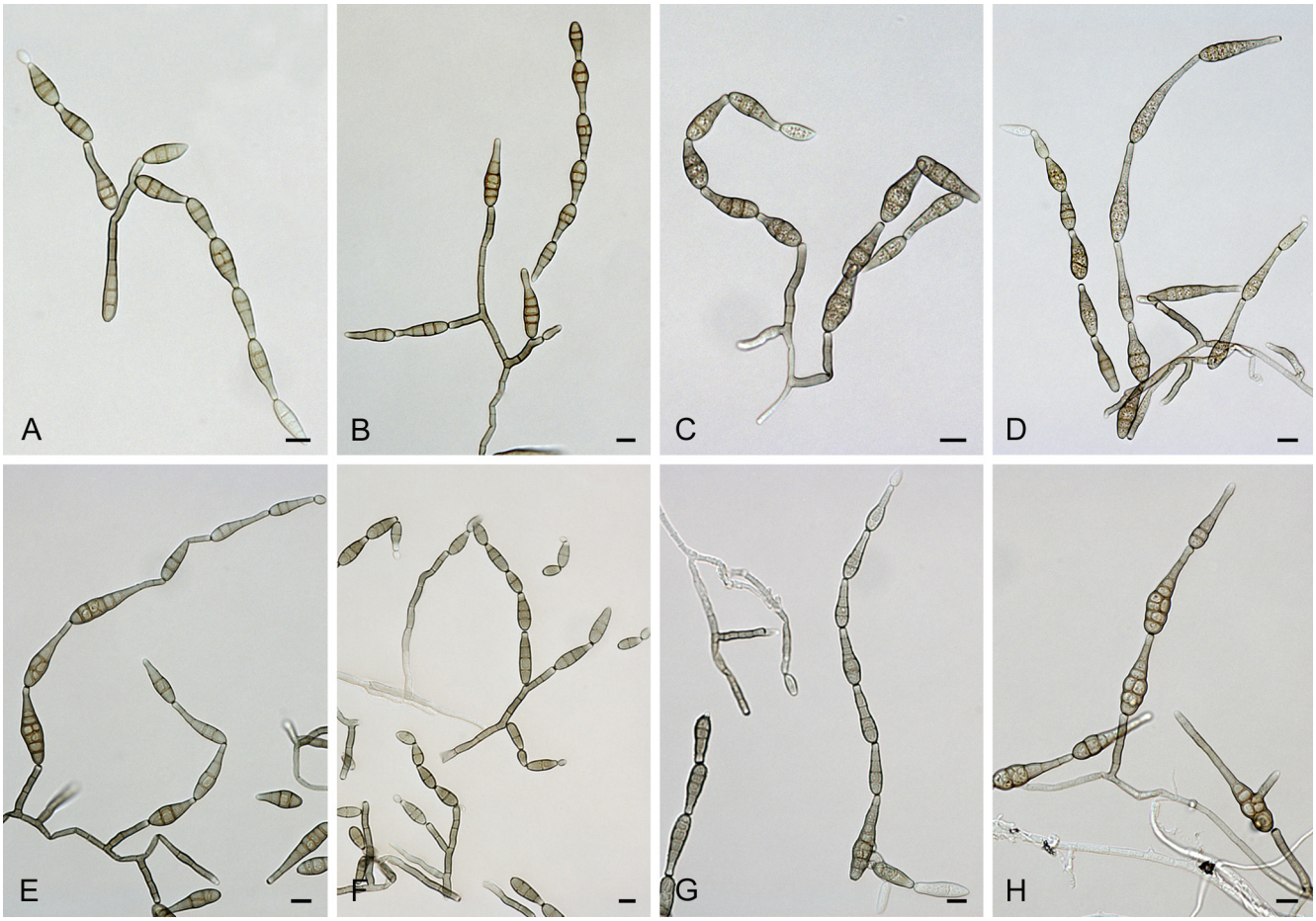


Fig. 4. *Alternaria gossypina* conidia and conidiophores. A–B. CBS 100.23. C–D. CBS 104.32. E–F. CBS 107.36. G–H. CBS 102597. Scale bars = 10 µm.

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 easily be distinguished from *A. alternata* using molecular data. 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).

Notes: Although *A. longipes* only has a few unique fixed nucleotides, the species can easily be distinguished from *A. alternata* using molecular data. 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.

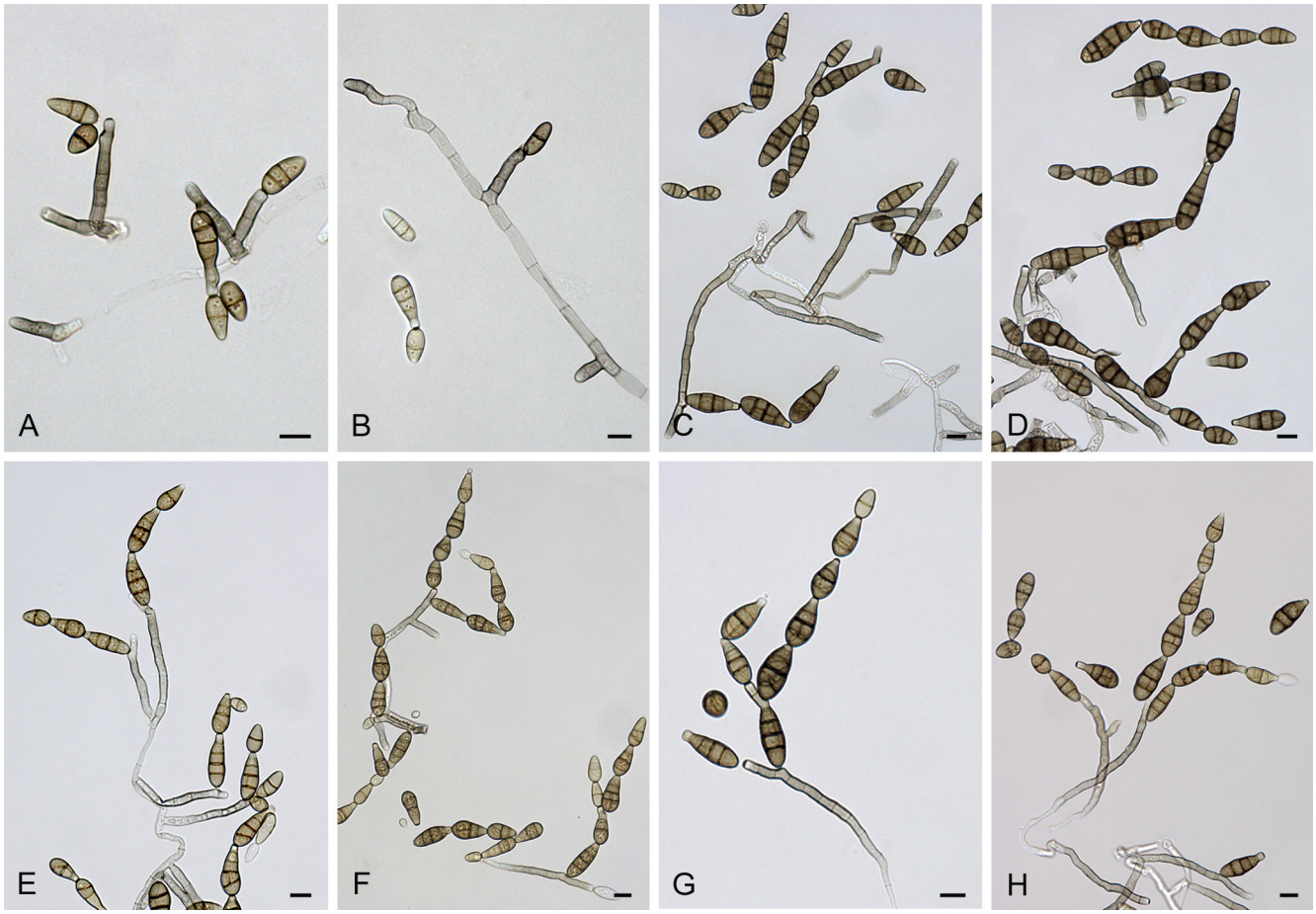


Fig. 5. *Alternaria arborescens* species complex conidia and conidiophores. A–B. *A. geophila* CBS 101.13. C–D. *A. arborescens* CBS 102605. E–F. *A. cerealis* CBS 119544. G–H. *A. senecionicola* CBS 119545. Scale bars = 10 µm.

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 (T).

Notes: Although *A. tomato* only has a few unique fixed nucleotides, the species can easily be distinguished from *A. alternata* using molecular data. 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.
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 **ex-type** 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 (G), 504 (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, the well-known name *A. arborescens* is retained above the relatively unknown name *A. geophila* for the species complex. The morphospecies present in this complex could not be resolved 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, priority of the name *A. arborescens* over *A. geophila* is strongly suggested. The isolate CBS 126.60 was deposited in the CBS collection as *A. maritima*; however, the type material of *A. maritima* is unknown, and therefore *A. maritima* is not included within the AASC pending the recollection of suitable material of *A. maritima*.

DISCUSSION

The aim of the present study was to employ genome comparisons and molecular phylogenies to clarify the species present in

Alternaria sect. *Alternaria*. The *Alternaria* genomes generated in this study ranged in size from 32.0–39.1 Mb (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* (~12 % repeats) and *A. alternantherae* (~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 ~5 % of repeats (Table 2). The percentage of repeats within sect. *Alternaria* is relatively low, 1.4–2.7 %, with the highest percentage of repeats in the *A. arborescens* genome. The isolates which are now named *A. alternata*, only ranged from 1.4–1.7 %. The genome assembly shows a high similarity between the isolates within sect. *Alternaria*; 96.7–98.2 % genome identity within sect. *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 sect. *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 phylogenetic species boundaries proposed here for sect. *Alternaria* are corroborated by the percentage of SNPs found in both the genome and transcriptome studies. The morphospecies 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.

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 *A. alternata* (Akimitsu *et al.* 2014), two are now recognised as separate phylogenetic species in sect. *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. sp.*, which affect tangerine and / or rough lemon, respectively (Masanuka *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, Masanuka *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 *et al.* (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*. Both *endoPG* and OPA10-2 are used in the multi-gene phylogeny presented here, but could only distinguish 11 species of the 52 morphospecies previously described. Also, the molecular phylogenies obtained from the 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 sect. *Alternaria* and the low SNP count derived by the genomic and transcriptomic data between isolates of sect. *Alternaria* led to the conclusion to synonymise 35 *Alternaria* morphospecies under *A. alternata*. As mentioned above, the detection of host-specific toxins could eventually give rise to several new *formae speciales* of *A. alternata*.

In a later study the same authors (Stewart *et al.* 2014) estimated the evolutionary histories of four nuclear loci on a worldwide sample of *A. alternata* isolates, causing citrus brown spot, using the coalescent theory. Next to the phylogenetic species concepts for estimating the species boundaries, two approaches were used that incorporate uncertainty in gene genealogies when lineage sorting and non-reciprocal monophyly of gene trees is common. The coalescent analyses revealed that the phylogenetic lineages are strongly influenced by incomplete lineage sorting and recombination. Also a study of the mating system of *A. alternata* isolates causing citrus brown spot found signatures of recombination (Stewart *et al.* 2013b). Andrew *et al.* (2009) already hypothesised that recombination and incomplete lineage sorting could explain the significant incongruence they found among gene genealogies in a four-gene species phylogeny on small-spored *Alternaria*, and the several putative recombination events that were identified within two non-coding regions. In agreement with our findings, little support was found for most of the morphospecies, when using these quantitative species recognition approaches.

Most of the synonymised morphospecies (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 known, no new isolates of these species were 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. sp.* of *A. alternata*. Nine of the synonymised morphospecies 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 causal agent 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 with molecular data. 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 only 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 arborescent-like 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 phylogenetic species present in this complex. Next to the known pathogenicity of *A. arborescens* on tomato, caused by the production of the AL-toxin, 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 postharvest 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 previously recognised as being of medical importance. 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 sect. *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 need for this research is stressed by examining recent publications on *Alternaria* spp. from sect. *Alternaria*. Two *Alternaria* species that were both argued as new based on phylogenetic data, and 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* sect. *Alternaria* treated in recently published manuscripts that need to be confirmed by, or subjected to, a multilocus sequence analysis in light of the present study. The research presented here will hopefully make the correct identification of species in sect. *Alternaria* easier for other researchers confronted with these species.

CONCLUSIONS

Based on genome comparisons and molecular phylogenies, *Alternaria* sect. *Alternaria* consists 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 sect. *Alternaria*, but the inconsistencies in morphology and molecular data makes further research necessary. By providing guidelines for the naming and identification of phylogenetic species in *Alternaria* sect. *Alternaria*, a stable and consistent taxonomic treatment of this section can hopefully be accomplished for the future. 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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