Ph. D. Thesis

Integrated species recognition of the genus *Alternaria*

(統合的概念による*Alternaria*属菌の種の類別)

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Chapter 1

General introduction

GENERAL INFORMATION AND REVIEW OF PLANT DISEASES CAUSED BY *ALTERNARIOID* SPECIES IN JAPAN

Alternaria Nees is an anamorphic *Ascomycota* (*Pleosporaceae*, *Pleosporales*) genus characterized by phaeodictyospores or phaeophragmospores (Seifert *et al.* 2011) (Fig. 1.1A). *Alternaria* species are generally ubiquitous fungi in the atmosphere and are commonly found on both living and dead plants; they can be saprophytic in litter and soil, endophytic in plant leaves and seeds, and pathogenic (Guo *et al.* 2004; Kirk *et al.* 2008). Most species are recognized as important plant pathogens chiefly on herbaceous plants (Yu 2001), but some are also allergenic and can cause mycoses of humans and insects (Rossmann *et al.* 1996; Christias *et al.* 2001; Downs *et al.* 2001; Hoog & Horré 2002).

As plant pathogens, *Alternaria* species usually cause circular leaf spots and spread to nearby plants through conidia produced on lesions, but their economic importance is primarily related to their seed-borne phases (Groves & Skolko 1944; Neergaard 1945; Richardson 1990; Tohyama 1993; Rathod 2012). In particular, two pathogenic species on carrot, *Alternaria dauci* (J.G. Kühn) J.W. Groves & Skolko and *Alternaria radicina* Meier, Drechsler & E.D. Eddy, are regarded as important seed-borne pathogens (Farrar *et al.* 2004), and the International Seed Testing Association (ISTA) published validated seed health testing methods for these pathogens, which include alternative methods developed by the International Seed Health Initiative for Vegetable Crops, ISF (ISHI-Veg). Under these rules, 400 sample seeds per lot are recommended

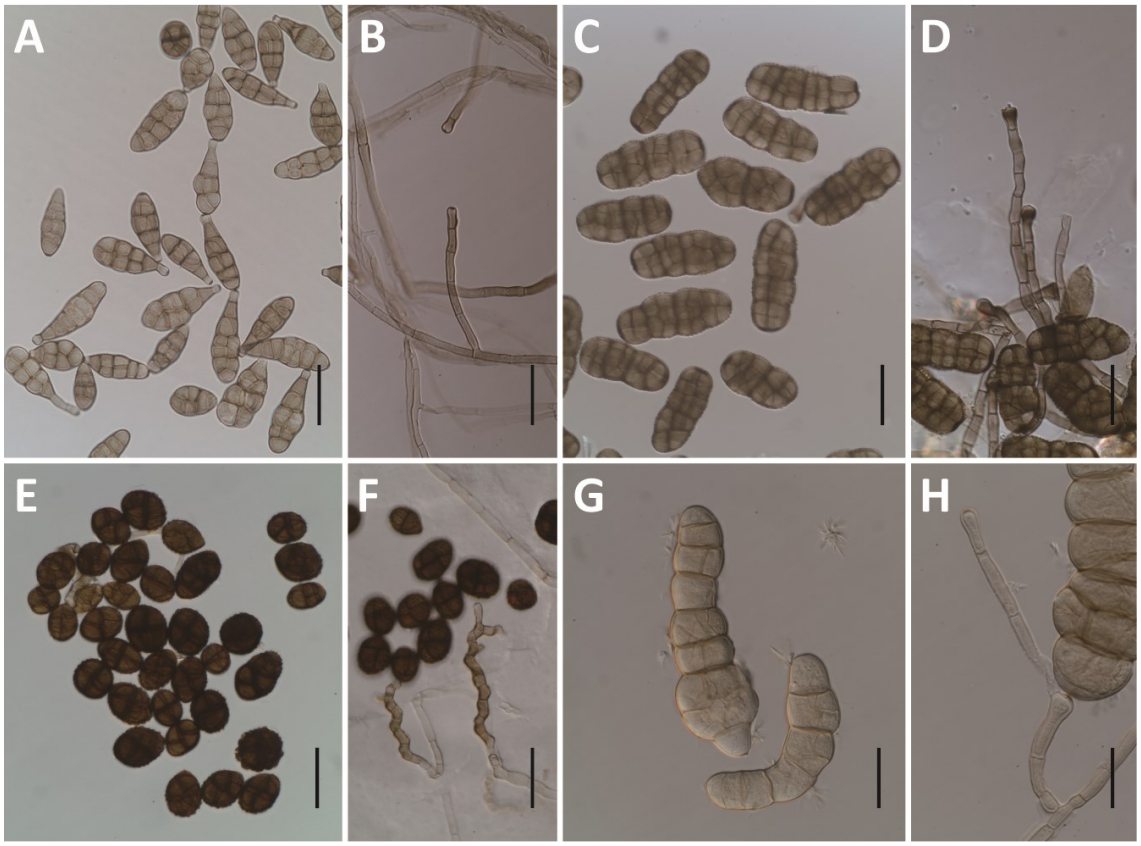

Fig. 1.1. Morphological characteristics of the genus *Alternaria* and allied genera. **A–B.** *Alternaria gaisen*. **C–D.** *Stemphylium vesicarium*. **E–F.** *Alternaria atra* (= *Ulocladium atrum*). **G–H.** *Alternariaster helianthi*. Bars = 25 μm.

to ensure reliable results (ISTA 2018). On brassicaceous plants, *Alternaria brassicae* (Berk.) Sacc., *Alternaria brassicicola* (Schwein.) Wiltshire, and *Alternaria japonica* Yoshii are the most common and important seed-borne pathogens (Maude & Humpherson-Jones 1980; Tohyama & Tsuda 1995; Rude *et al.* 1999; Shrestha *et al.* 2000; Nishikawa *et al.* 2014). While all three species are commonly distributed in Japan (Katumoto 2010), their frequencies on seeds were vary; *A. brassicae* has never been detected from seeds in Japan but is common in Europe, and *A. brassicicola* is more common on *Brassica oleracea* L. than *B. rapa* L. while *A. japonica* shows the reverse pattern (Nishikawa *et al.* 2014). In this way, *Alternaria* species have been

recognized as important seed-borne fungi and PCR-based detection methods for seeds have been developed to establish a more sensitive testing method (Pryor & Gilbertson 2001; Iacomi-Vasilescu *et al.* 2002; Konstantinova *et al.* 2002; Guillemette *et al.* 2004), but their ecological characteristics such as the seed-borne pathway and host preference of each pathogen are still unclear.

Regarding the biodiversity and distribution of *Alternaria* in Japan, the latest records of plant diseases caused by Alternarioid fungi in Japan include 146 pathogen–plant genus combinations based on the NIAS Genebank database of plant diseases in Japan (https://www.gene.affrc.go.jp/databases-micro_pl_diseases_en.php) (Table 1.1). However, the pathogens in 36 records (approximately 25 % of the records) remain unidentified, and the current taxonomy of alternarioid species has not been verified since it was published in Yamamoto (1960). Moreover, the list of acceptable epithets consists of only around 50 names, which may represent less than 25 % of the hitherto known species in the world. Thus, it can be concluded that the diversity of Japanese species of the genus *Alternaria* is unknown, and therefore a comprehensive taxonomic re-examination is needed.

HISTORY OF TAXONOMY AND ITS PROBLEMS

Alternaria Nees, *Macrosporium* Fries, *Stemphylium* Wallroth, and *Ulocladium* Preuss were established in the 1800s and are regarded as related genera characterized by phaeodictyospores (Fig. 1.1A–F). The genus *Alternaria* was originally typified by *Alternaria tenuis* Nees, but now *Alternaria alternata* (Fr.) Keissl. is regarded as the legitimate name of the type species (Nees 1816; Keissler 1912; Simmons 1967). A detailed taxonomic history of allied genera including *Thyrospora* Tehon & Daniels and the type of the genus *Alternaria* have been described in Neergaard (1945), Nishimura (1980a), Simmons (2007), and Woudenberg *et al.* (2013). Simmons (1967) redefined *Alternaria*, *Stemphylium*, and *Ulocladium* based on their mode of conidiogenesis, and designated the neotype of *A. alternata*. Two additional genera,

Table 1.1. Plant diseases caused by Alternarioid fungi in Japan.

Pathogen	Current / pleasant name of pathogen	Host genera
Alternaria alstroemeriae E.G. Simmons & C.F. Hill		*Alstroemeria*
Alternaria alternata (Fr.) Keissl.		*Abelmoschus*, *Alstroemeria*, *Amygdalus*, *Arachis*, *Beta*, *Carthamus*, *Chamaecyparis*, *Cucumis*, *Echinacea*, *Echinops*, *Euphorbia*, *Eustoma*, *Gentiana*, *Impatiens*, *Ipomoea*, *Medicago*, *Morinda*, *Nicotiana*, *Ocimum*, *Oryza*, *Pelargonium*, *Populus*, *Pyrus*, *Rosa*, *Salvia*, *Solanum*, *Spinacia*, *Symphoricarpos*, *Vaccinium*
Alternaria alternata (Fr.) Keissl. (tomato pathotype)	*Alternaria arborescens* E.G. Simmons	*Solanum*
Alternaria alternata (Fr.) Keissl. (strawberry pathotype)	*Alternaria gaisen* Bokura f. sp. *fragariae*	*Fragaria*
Alternaria alternata (Fr.) Keissl. (tobacco pathotype)	*Alternaria longipes* (Ellis & Everh.) E.W. Mason	*Nicotiana*
Alternaria azukiae (Hara) Hara	*Alternaria azukiae* (Hara) Miura	*Vigna*
Alternaria bataticola Ikata ex W. Yamam.		*Ipomoea*
Alternaria brassicae (Berk.) Sacc.		*Armoracia*, *Brassica*, *Eutrema*, *Linum**, *Raphanus*
Alternaria brassicae f. sp. *phaseoli**		*Phaseolus*
Alternaria brassicicola (Schwein.) Wiltshire		*Brassica*, *Raphanus*
Alternaria calendulae W. Yamam.	*Alternaria calendulae* Ondřej	*Calendula*
Alternaria catalpae (Ellis & Martin) Parker	*Alternaria catalpae* (Ellis & G. Martin) P. Joly	*Catalpa*
Alternaria cerasi Potebnia		*Cerasus*
Alternaria cheiranthi (Lib.) P.C. Bolle		*Brassica*
Alternaria cinerariae Hori & Enjoji		*Farfugium*, *Pericallis*, *Senecio*

Table 1.1. (Continued).

Pathogen	Current name / proposal name of pathogen	Host genera
Alternaria citri Ellis & N. Pierce	*Alternaria alternata* (Fr.) Keissl.	*Citrus*, *Phoenix*
Alternaria crassa (Sacc.) Rands		*Datura*
Alternaria cucumerina (Ellis & Everh.) J.A. Elliott		*Cucurbita*
Alternaria dauci (J.G. Kühn) J.W. Groves & Skolko		*Daucus*
Alternaria dianthi F. Stevens & J.G. Hall	*Alternaria nobilis* (Vize) E.G. Simmons	*Dianthus*
Alternaria fasciculata (Cooke & Ellis) L.R. Jones & Grout	*Alternaria alternata* (Fr.) Keissl.	*Phaseolus*, *Pisum*
Alternaria gomphrenae Togashi		*Gomphrena*
Alternaria gossypii (Jacz.) Y. Nisik., K. Kimura & Miyaw.		*Gossypium*
Alternaria gossypina (Thüm.) J.C.F. Hopkins		*Gossypium*
Alternaria helianthi (Hansf.) Tubaki & Nishih.	*Alternariaster helianthi* (Hansf.) E.G. Simmons	*Helianthus*
Alternaria iridicola (Ellis & Everh.) J.A. Elliott		*Iris*
Alternaria japonica Yoshii		*Brassica*, *Matthiola*, *Orychophragmus*, *Raphanus*
Alternaria kikuchiana S. Tanaka	*Alternaria gaisen* Bokura f. sp. *pyri*	*Pyrus*
Alternaria longissima Deighton & MacGarvie	*Prathoda longissima* (Deighton & MacGarvie) E.G. Simmons*	*Petunia*
Alternaria macrospora Zimm.		*Gossypium*
Alternaria mali Roberts	*Alternaria alternata* (Fr.) Keissl. f. sp. *mali*	*Malus*
Alternaria manshurica Hara		*Pyrus*
Alternaria nelumbii (Ellis & Everhart) Enlows & F.V. Rand	*Alternaria nelumbii* Enlows & F.V. Rand	*Nelumbo*
Alternaria oryzae Hara		*Oryza*

Table 1.1. (Continued).

Pathogen	Current name / proposal name of pathogen	Host genera
Alternaria panax Whetzel		*Aralia*, *Panax*, *Polyscias*
Alternaria petasitis M. Kubota, Kishi & Abiko		*Petasites*
Alternaria petroselini (Neerg.) E.G. Simmons		*Petroselinum*
Alternaria porri (Ellis) Cif.		*Allium*
Alternaria radicina Meier, Drechsler & E.D. Eddy		*Daucus*
Alternaria ricini (Yoshii) Hansf.		*Ricinus*
Alternaria sesami (E. Kawam.) Mohanty & Behera*		*Sesamum*
Alternaria sesamicola E. Kawam.*		*Sesamum*
Alternaria solani Sorauer		*Capsicum*, *Glebionis**, *Solanum*
Alternaria somniferi (Hariot & Broome) Sawada	*Alternaria somniferi* (Har. & Briard) Sawada	*Papaver*
Alternaria sp.		*Allium*, *Amaranthus*, *Anemone*, *Aralia*, *Aronia*, *Beta*, *Callistephus*, *Cannabis*, *Cerasus*, *Coreopsis*, *Cosmos*, *Cryptomeria*, *Dianthus*, *Euphorbia*, *Firmiana*, *Glycine*, *Gypsophila*, *Hibiscus*, *Larix*, *Malus*, *Nicotiana*, *Oryza*, *Paeonia*, *Primula*, *Pueraria*, *Pyrus*, *Tithonia*, *Triticum*, *Zea*
Alternaria sp.	*Alternaria cinerariae* Hori & Enjoji	*Gynura*
Alternaria spinaciae Allesch. & F. Noack		*Spinacia*
Alternaria steviae Ishiba, T. Yokoy. & Tani**		*Stevia*
Alternaria tagetica S.K. Shome & Mustafee		*Tagetes*
Alternaria tenuissima (Nees) Wiltshire	*Alternaria alternata* (Fr.) Keissl.	*Celosia*, *Cosmos*, *Gerbera*, *Lunaria*, *Vicia*
Alternaria tomato (Cooke) Weber	*Alternaria tomato* (Cooke) L.R. Jones	*Solanum*
Alternaria violae L.D. Galloway & Dorsett		*Viola*

Table 1.1. (Continued).

Pathogen	Current name / proposal name of pathogen	Host genera
Alternaria zinniae H. Pape	*Alternaria zinniae* H. Pape ex M.B. Ellis	*Zinnia*
Embellisia allii (Campan.) E.G. Simmons	*Alternaria embellisia* Woudenberg & Crous	*Allium*
Embellisia hyacinthi de Hoog & P.J. Mull. bis	*Alternaria hyacinthi* (de Hoog & P.J. Muller bis) Woudenb. & Crous	*Hyacinthus*
Macrosporium abutilonis Speg.	*Alternaria abutilonis* (Pass.) Schwarze	*Abutilon*
Macrosporium cladosporioides Desm.*		*Trifolium*
Macrosporium nigricantium G.F. Atk.	*Stemphylium nigricans* (G.F. Atk.) E.G. Simmons	*Gossypium*
Macrosporium nobile Vize	*Alternaria nobilis* (Vize) E.G. Simmons	*Dianthus*
Macrosporium pirorum Cooke*	*Macrosporium pyrorum* Cooke*	*Pyrus*
Macrosporium sp.*		*Amorpha*, *Citrus*, *Eriobotrya*, *Lespedeza*, *Metasequoia*, *Pinus*
Nimbya scirpicola (Fuckel) E.G. Simmons	*Alternaria scirpicola* (Fuckel) Sivan.	*Eleocharis*
Pleospora papaveracea (De Not.) Sacc.	*Alternaria penicillata* (Corda) Woudenb. & Crous	*Papaver*
Trichoconiella padwickii (Ganguly) B.L. Jain		*Oryza*
Ulocladium sp.*		*Tetragonia*

Generated based on the NIAS database of plant diseases in Japan (updated 4 Sep. 2018).

* Doubtful pathogen or host names require further verification.

** Additional species not in the database of plant diseases in Japan.

Embellisia and *Nimbya*, subsequently established by Simmons (1971, 1989) were also recognized as related genera of *Alternaria* until recently. In the 1990s to early 2000s, Simmons and several researchers around the world continued to split alternarioid species and created many new genera such as *Alternariaster* E.G. Simmons, *Chalastospora* E.G. Simmons, *Crivellia* Shoemaker & Inderb., *Sinomyces* Yong Wang bis & X.G. Zhang, *Teretispora* E.G. Simmons, and *Undifilum* B.M. Pryor, Creamer, Shoemaker, McLain-Romero & Hambl. on the basis of conidial morphology and molecular phylogeny as members of the *Alternaria* complex (Zhang 2003; Inderbitzin *et al.* 2006; Simmons 2007; Pryor *et al.* 2009; Wang *et al.* 2011). The latest molecular phylogenies have drastically reconstructed the above allied genera, which were combined into one large genus and synonymized with *Alternaria* (Woudenberg *et al.* 2013).

Previous taxonomies based on morphology focused on not only conidial features (size, shape, beak, septum, and cell structure) and sporulation patterns (chain formation and branching) but also host substrates as species keys (Simmons 2007). These features, however, are often complicated for people other than experts in mycology, and have led to an increase of similar species and classifications that are confusing for plant pathologists; the problem of the taxonomy of seven known host-selective toxin (HST) producers, which were defined as pathotypes of *A*. *alternata* by Nishimura (1980b) whereas Simmons (1999) recognized them as distinct morphological species, has been argued in the past. Moreover, host-dependent classification (mostly at the rank of plant genus) has facilitated an unavoidable increase in the number of species without pathological descriptions, although it is easy to use for identifying of plant-pathogenic species. It is supposed that some of the host genera used as taxonomic keys for species identification may include false hosts because alternarioid fungi are commonly found on both living and dead plants, as I mentioned above. Even pathogenic species are often isolated from non-host substrates such as seeds, behaving like hitchhikers.

Simmons himself also tried to classify the enlarged and complicated genus into 14 species-groups (Simmons 1992, 2007; Simmons & Roberts 1993), but the classification system was factitious and determined by morphological features and was still confusing. This taxonomic grouping was followed by molecular phylogenetic analyses using a great number of

living cultures that included ex-type and reference isolates left out by E.G. Simmons, which resolved sections through the species-group concept (Pryor & Gilbertson 2000; Lawrence *et al.* 2013). Woudenberg *et al.* (2013, 2015) supported these sections and also synonymized the names of many morphological species based on genome and transcriptome comparisons and phylogenetic analyses of genome sequences generated by next-generation sequencing. While these molecular phylogenetic studies have helped to clarify the taxonomy of *Alternaria* and identify cryptic species, indistinguishable uncharacterized taxa in terms of both morphology and pathology have emerged.

OBJECTIVES AND OUTLINE OF THIS THESIS

The taxonomy of the genus *Alternaria* is basically based on conidial morphology, sporulation patterns, and differences of source plants (mostly at the rank of genus) or substrates (Simmons 2007). However, most species of the genus are ubiquitous and pleomorphic fungi (Rotem 1994; Seifert *et al.* 2011; Lawrence *et al.* 2016). This means their original source plants include false hosts, and consequently the genus *Alternaria* was expanded to include over 400 species (Simmons 2007). Despite the application of molecular phylogenetic analyses to the genus (Lawrence *et al.* 2013; Woudenberg *et al.* 2013), it still remains, partially at least, unclear what species constitute *Alternaria*. Therefore, the author considers phenotyping within the true host range to be important to distinguish species boundaries through a survey of alternarioid fungi distributed in Japan, and integrated species recognition based on morphology, molecular phylogeny, and pathogenicity is proposed in this study.

Chapter 1 is an introduction to the genus *Alternaria*. The objectives of this thesis are described with background such as the economic importance of the genus owing to their seedborne dispersal, the history of its taxonomy and the problems in clinical plant pathology that need to be resolved. Above all, the novelty and necessity of phenotyping within species host ranges, which is the principal method of species characterization in this thesis, are

highlighted.

Chapter 2 treats *Alternaria cinerariae* as an example of morphological variability within a species in the genus. The results allow reconsideration of which characters should be adopted as species keys in the genus *Alternaria*. The interesting host selectivity was also found for the species, and was used able to redefine *A. cinerariae*.

Chapter 3 provides a taxonomic revision of the strawberry black leaf spot pathogen, which remains an unclassified taxon among the seven known pathotypes of host-selective toxin (HST)-producing species belonging to the most morphologically confusable taxon, *Alternaria alternata*-like species. From morphological observations and a multi-locus molecular phylogeny, the causal pathogen was identified and re-described as *A. gaisen* Nagano ex Bokura, which should bring argument about the reclassification of the known pathotypes almost to an end.

Chapter 4 focuses on four newly recorded species of *Alternaria* in Japan. To enable phenotyping of these four species, which are new to Japan, *A. alstroemeriae*, *A. celosiicola*, *A. crassa*, and *A. petroselini*, an integrated species recognition method based on morphology, phylogenetic analysis using ITS sequences, and experimental host range was tested. The results show experimental host ranges well along host systematics for each pathogenic species, and provide evidence that integrated species recognition is useful to understand species boundaries of *Alternaria*.

Chapter 5 discusses the utility of phenotyping based on experimental host ranges to distinguish closely related species, namely species pathogenic on *Amaranthaceae*, *Brassicaceae*, *Apiaceae*, *Solanaceae*, and *Iridaceae*. A taxonomy of the Japanese *Alternaria* species examined in this thesis, which include 26 species covering 14 known *Alternaria* sections and two monotypic lineages, is also provided based on integrated species recognition. Consequently, three new species, *A. paragomphrenae*, *A. cylindrica* and *A. triangularis*, are described.

Chapter 6 discusses the biodiversity of Japanese *Alternaria* species and summarizes the achievements of this thesis. It is concluded that the integrated species recognition method proposed in this study can help make the taxonomy of plant pathogenic fungi more accurate

and convenient for both plant pathologists and mycologists, with an emphasis on the importance of phenotyping with pathogenicity.

The isolates examined in this study exclude *Stemphylium* species and *Alternariaster helianthi* (Hansf.) E.G. Simmons (*Leptosphaeriaceae*), which is not applicable to the genus *Alternaria* sensu Woudenberg *et al.* (2013), from whole collections (Fig. 1.1C, D, G, H).

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Chapter 2

Morphological variation and experimental host range of *Alternaria cinerariae*

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ABSTRACT

Alternaria cinerariae, an important and well-known plant pathogen of cineraria, has been delimited by its narrowly to broadly ellipsoid or ovoid conidia with blunt-tapered false beaks, but without descriptions of its morphological variability and host range. Leaf spot diseases have recently been found on asteraceous plants *Pericallis cruenta*, *Farfugium japonicum*, and *Gynura bicolor* in Japan, with *A. cinerariae*-like isolates subsequently obtained from the lesions. In this study, we identified these isolates as *A*. *cinerariae* using an integrated species criterion based on morphology, phylogeny, and pathogenicity, and examined their morphological variability. Although isolates exhibited morphological differences with respect to sporulation pattern, conidial swelling, and chlamydospore formation, phylogenetic analysis using *gapdh*, *rpb2*, and *tef1* sequences clustered them together in a single clade with a previously recognized *A. cinerariae* strain. Inoculation tests on 17 species, including five *Senecioneae* species, were performed to determine the experimental host range of *A*. *cinerariae*. The results suggest that *A*. *cinerariae* has considerable morphological variation and preferential pathogenicity to *Senecioneae* plants, and also indicate that *G. bicolor* should be added as a natural host of *A*. *cinerariae*.

Keywords: Host specificity, Intraspecific variation, Pathogenicity, Phylogeny

INTRODUCTION

Alternaria cinerariae Hori & Enjoji was first described from central Japan (Enjoji 1931) as a pathogen of *Senecio cruentus* (Masson ex L'Hér.) DC. (*Asteraceae*), and is now widely distributed in Europe, the United States, Korea, and New Zealand (Neergaard 1945; Ellis 1976; Simmons 1997; Yu 2001; Farr & Rossman 2014). *Alternaria senecionis* Neerg. was described as a new species on *S. cruentus* (Neergaard 1945), but was later placed in synonymy with *A. cinerariae* (Joly 1964). On account of a well-known Latin translation by Yamamoto (1960) of the original Japanese description of *A. cinerariae*, this is a validly published species name based on the 1931 publication (Joly 1964; Yu 2001; Simmons 2007). Morphologically, *A. cinerariae* is well characterized by narrowly to broadly ellipsoid or ovoid conidia with a blunt-tapered false beak. Based on this morphology and the latest phylogenetic studies, the species is classified into the sonchi species-group (Simmons 1997; Hong *et al.* 2005; Lawrence *et al.* 2012), now known as *Alternaria* sect. *Sonchi* D.P. Lawr. *et al*. (Lawrence *et al.* 2013; Woudenberg *et al.* 2013).

Alternaria cinerariae is usually observed on *Senecio* spp., including *S. cruentus* and *S. cineraria* DC. (Takano 2001). It has therefore generally been believed that the pathogenicity of this species is restricted to the genus *Senecio* (Yu 2001). *Senecio cruentus* and *S. cineraria* have more recently been reassigned to different genera, as *Pericallis cruenta* (Masson ex L'Hér.) Bolle and *Jacobaea maritima* (L.) Pelser & Meijden, respectively, and are not closely related to *Senecio* (Pelser *et al.* 2007). In addition, two other asteraceous genera—*Farfugium* [*F. japonicum* (L.) Kitam.] and *Ligularia* (*Ligularia* sp.)—were recently added to the list of *A. cinerariae* host genera (Simmons 1997, 2007; Sakoda *et al.* 2010). Interestingly, these host genera are all members of *Senecioneae*—the largest tribe of *Asteraceae*, with more than 150 genera (Pelser *et al.* 2007). Based on the most recent phylogenetic placements of these hosts (Pelser *et al.* 2007), the natural host range of *A. cinerariae* extends beyond a single genus, and may encompass the entire tribe.

Following these reports, we collected several large-spored *Alternaria* isolates from three *Senecioneae* plants, and compared their morphological and molecular characteristics. With

respect to conidial morphology, the collected isolates resembled one another and were regarded as members of the sonchi species-group, but were not completely identical to *A. cinerariae* from previous descriptions (Enjoji 1931; Neergaard 1945; Sobers 1964; Simmons 1997). Although detailed numerical descriptions of the inflated conidia are not currently available, this type of within-species morphological variation is often attributed to cell hypertrophy (Simmons 1997, 2007). Variation in conidial morphology of both small- and large-spored *Alternaria* species, including *A. alternata* (Fr.) Keissl. and *A. panax* Whetzel, is well known to occur among isolates and among different cultural condition (Misaghi *et al.* 1978; Yu *et al.* 1984).

Based on these observations, we speculated that *A. cinerariae* has host tribe-specific pathogenicity on *Senecioneae* along with considerable morphological variation. In this study, we attempted to verify this hypothesis. We also examined species delimitation of *A. cinerariae* using an integrated species criterion based on morphological characters, host ranges, and phylogenetic relationships.

MATERIALS AND METHODS

Fungal collection and isolation

Leaves showing leaf spot symptoms were collected from *P. cruenta* in Chiba Prefecture, Japan, and from *F. japonicum* and *Gynura bicolor* (Roxb. ex Willd.) DC. in Ibaraki Prefecture, Japan. Alternarioid conidia on spots of diseased leaves were suspended in sterilized water and spread on 2 % aqueous agar medium using a flame-sterilized microspatula. After incubation at 20 °C for 24 h, individual germinating conidia were confirmed under a light microscope and transferred to potato–carrot agar (PCA; Simmons 2007) using a flame-sterilized microtube (Nakashima *et al.* 2011). Purified cultures originating from single conidia derived from *P. cruenta* (AC3; Senecio isolate), *F. japonicum* (TY36 = AC57; Farfugium isolate), and *G. bicolor* (TY37 = AC58; Gynura isolate) were deposited as MAFF 243059, MAFF 241266, and MAFF

241267, respectively, in the Genebank, National Institute of Agrobiological Sciences, Tsukuba, Japan.

Morphological observation

For microscopic observations, the isolates were newly plated onto V8 juice agar (V8; Simmons 2007) and PCA. To obtain diagnostic conidia and conidial catenation, sporulation was induced according to methods in Nishikawa & Nakashima (2013). Briefly, plates were maintained at 25 °C in the dark for 7–14 d. The growing colonies were scratched and spread using a flame-sterilized microspatula, and aerial mycelia were removed to observe sporulation more easily. After 12–24 h of pre-incubation in unsealed Petri dishes at 25 °C under blacklight blue bulbs, the plates were incubated at 20 °C in the dark, and sporulation patterns and conidial morphology were observed once a day for 7 d. Conidia and conidiophores that had formed on the medium were mounted with Shear's mounting fluid [300 mL aqueous potassium acetate (2 %), 120 mL glycerin, and 180 mL ethanol (95 %)] and observed under a light microscope. The morphology of 100 conidia and 30 conidiophores was examined at 400× magnification.

Culture characteristics on potato-dextrose agar

Each isolate was plated onto potato-dextrose agar (PDA; 40 g potato, 20 g dextrose, and 20 g agar in 1000 mL distilled water) plates. After incubation in the dark for 7 d at 25 °C, five colonies of each isolate were measured and culture characteristics were recorded.

Inoculation tests

Conidia that artificially sporulated on V8 medium were washed with sterile distilled water containing 0.02 % polyoxyethylene (20) sorbitan monolaurate (Wako Pure Chemicals, Osaka, Japan) and rubbed with a sterilized plastic spreader. Released conidia were harvested and used as inocula (Nishikawa & Nakashima 2013). Concentrations of conidial suspensions of Senecio, Farfugium, and Gynura isolates were adjusted to a range of 4.1–6.4 × 10^4 conidia/mL. To determine experimental host range, each inoculum was sprayed onto mature leaves of potted

plants (at least 3–5 replicates) of 17 species (Table 2.1). The 17 species comprised 15 species of *Asteraceae*, including the five *Senecioneae* species, and two species previously reported as experimental hosts (Neergaard 1945): *Cucumis sativus* L. (*Cucurbitaceae*) and *Clarkia amoena* (Lehm.) Nels. & Macbr. (*Onagraceae*). All inoculated plants were maintained in an incubator under moist conditions at 20 °C.

Virulent phenotypes were evaluated 10 d post-inoculation (dpi) using the scale described by Chaerani *et al.* (2007) (0: no visible leaf lesions; 1: up to 10 % of leaf area affected; 2: 11–25 %; 3: 26–50 %; 4: 51–75 %; and 5: more than 75 % of leaf area affected or the leaf abscised), and the means were calculated as disease severity. Significant differences of disease severity means among inoculated plants for each isolate were statistically evaluated by Ryan's test (Day & Quinn 1989) at $P = 0.05$. We also observed changes in lesion diam and presence or absence of sporulation on the lesion to evaluate pathogenicity. Symptoms were continuously observed until 30 dpi.

DNA extraction and phylogenetic analyses

Total DNA was extracted from mycelia in / on agar discs using UltraClean Microbial DNA Isolation kits (Mo-Bio Laboratories, Carlsbad, CA, USA). Three protein-coding genes—*gapdh*, *rpb2*, and *tef1*—were amplified using *Taq* DNA polymerase (Bioline, London, UK) and primers gpd1 and gpd2 (Berbee *et al.* 1999) for *gapdh*, RPB2-6F and fRPB2-7cR for *rpb2* (Liu *et al.* 1999), and EF1-728F and EF1-986R for *tef1* (Carbone & Kohn 1999) in a T100 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) as described by Woudenberg *et al.* (2013). Both strands were sequenced using a BigDye Terminator v.3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems, Carlsbad, CA, USA) in a T100 thermal cycler. The products were purified with Sephadex G-50 medium (GE Healthcare, Buckinghamshire, UK) and multiscreen plates (Millipore, Billerica, MA, USA; Crous *et al.* 2010). Sequences were analyzed on a 3730xl Genetic Analyzer (Applied Biosystems) at the Life Science Research Center, Mie University, Mie, Japan. Sequences were deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers AB862967–AB862984 and AB906670 (Table 2.2). In addition, sequences of three genes (*act*,

Table 2.1. Host ranges of Japanese isolates of *Alternaria cinerariae* in inoculation tests.

Higher taxon	Plant species	Disease severity[a] of isolates obtained from:			Lesion diameter[b]	Notes
		Pericallis (MAFF 243059)	*Farfugium* (MAFF 241266)	*Gynura* (MAFF 241267)		
Asteraceae: Asteroideae, Senecioneae, Othonninae	*Euryops pectinatus*	0.8 b*	4.2 ab	4.0 a	+, ±	Non- or strongly pathogenic depending on isolate: black spots to leaf blight with rich sporulation by 8–10 dpi (MAFF 241266 and MAFF 241267); small spots rare by 30 dpi without sporulation (MAFF 243059).
Senecioninae	*Gynura bicolor*	4.5 a	4.8 a	4.5 a	+	Strongly pathogenic; distinct to indistinct spots, rich sporulation, defoliation by 7 dpi.
	Jacobaea maritima (*Senecio cineraria*)	4.3 a	4.7 ab	4.5 a	+	Pathogenic; black spots to leaf blight, mycelial rot with rich sporulation by 5–7 dpi.
	Pericallis cruenta (*Senecio cruentus*)	4.0 a	4.7 ab	5.0 a	+	Pathogenic; indistinct black spots to leaf blight, rich sporulation by 8 dpi.
Tussilagininae	*Farfugium japonicum*	3.7 a	3.7 ab	4.0 a	+	Pathogenic; distinct spots, rich sporulation by 10 dpi.
Anthemideae	*Glebionis coronaria*	0 c	0 c	0 c	−	Non-pathogenic; no symptoms or sporulation by 30 dpi.
Astereae	*Callistephus chinensis*	0.7 bc	1.2 bc	1.5 b	±	Non- to weakly pathogenic; distinct spots by 7 dpi and weak spots by 30 dpi, sometimes with sporulation.
Coreopsideae	*Cosmos bipinnatus*	2.3 ab	3.1 b	3.8 ab	+	Pathogenic; distinct spots to leaf blight by 3 dpi due to pinnate leaves, sporulation by 7 dpi.
	Cosmos sulphureus	0.3 bc	1.8 b	1.8 b	±	Non-pathogenic; spots rare by 10 dpi and weak by 30 dpi, no sporulation.
Heliantheae	*Zinnia elegans*	0.2 bc	0.2 bc	1.0 bc	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.
Tageteae	*Tagetes patula*	0 c	0.2 bc	0.5 bc	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.
Cichorioideae, Cichorieae, Lactucinae	*Lactuca sativa*	0.2 bc	0.3 bc	0.8 bc	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.
Crepidinae	*Taraxacum officinale*	0.1 bc	0.4 bc	0.6 bc	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.

Table 2.1. (Continued).

Higher taxon	Plant species	Disease severity[a] of isolates obtained from:			Lesion diameter[b]	Notes
		Pericallis (MAFF 243059)	*Farfugium* (MAFF 241266)	*Gynura* (MAFF 241267)		
Carduoideae	*Centaurea cyanus*	2.3 ab	2.7 b	3.0 ab	+	Weakly pathogenic; distinct spots to leaf blight by 9 dpi, poor sporulation by 30 dpi.
Mutisioideae	*Gerbera hybrida*	0.2 bc	0.3 bc	1.0 b	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.
Cucurbitaceae	*Cucumis sativa*	0 c	0.5 bc	0.8 bc	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.
Onagraceae	*Clarkia amoena*	0 c	0 c	0 c	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.

[a] Mean disease severity at 9 d post-inoculation (dpi) rated on a 0–5 scale (0 = no visible lesions, 1 = <10 % leaf area affected, 2 = 11–25 %, 3 = 26–50 %, 4 = 51–75 %, and 5 = >75 % or defoliated).

[b] Change within 30 dpi; +, increased; ±, slightly increased; −, unchanged.

[*] Numbers within a column followed by the same letter are not significantly different according to Ryan's test at $P = 0.05$.

Table 2.2. Isolates used for the phylogenetic analyses in this study and their DDBJ accession numbers.

Species name	Section of *Alternaria*[a]	Strain number[b]	Host plant	DDBJ accession number[c]		
				gapdh	*rpb2*	*tef1*
Alternaria brassicae	-	AC29	*Brassica rapa*	**AB862967**	**AB862973**	**AB862979**
Alternaria brassicae	-	CBS 116528	*Brassica oleracea*	KC584102	KC584382	KC584641
Alternaria brassicicola	*Brassicicola*	AC70	*Raphanus sativus* var. *sativus*	**AB862968**	**AB862974**	**AB862980**
Alternaria brassicicola	*Brassicicola*	AC56	*Brassica rapa* subsp. *rapa*	**AB862969**	**AB862975**	**AB862981**
Alternaria brassicicola	*Brassicicola*	CBS 118699	*Brassica oleracea*	KC584103	KC584383	KC584642
Alternaria cinerariae	*Sonchi*	CBS 116495	*Ligularia* sp.	KC584109	KC584389	KC584648
Alternaria cinerariae[d]	*Sonchi*	MAFF 243059	*Pericallis cruenta*	**AB906670**	-	-
Alternaria cinerariae	*Sonchi*	MAFF 241266	*Farfugium japonicum*	**AB862970**	**AB862976**	**AB862982**
Alternaria cinerariae	*Sonchi*	MAFF 241267	*Gynura bicolor*	**AB862971**	**AB862977**	**AB862983**
Alternaria panax	*Panax*	MAFF 243161	*Polyscias fruticosa*	**AB862972**	**AB862978**	**AB862984**
Alternaria panax	*Panax*	CBS 482.81	*Aralia racemosa*	KC584128	KC584417	KC584675
Alternaria porri	*Porri*	CBS 116698	*Allium cepa*	KC584132	KC584421	KC584679
Alternaria radicina	*Radicina*	CBS 245.67	*Daucus carota*	KC584133	KC584423	KC584681
Alternaria sonchi	*Sonchi*	CBS 119675	*Sonchus asper*	KC584142	KC584433	KC584691

[a] Woudenberg *et al*. (2013).

[b] AC: Personal collection of the author; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; MAFF: Genebank, National Institute of Agrobiological Sciences, Tsukuba, Japan.

[c] Bold accession numbers were generated in this study.

[d] Additional sequences (*act*, *Alt a 1*, and ITS) were also deposited under accession numbers AB906672, AB906671, and AB906673, respectively.

Alt a 1, and ITS) of the Senecio isolate MAFF 243059 were obtained following the procedure described in Nishikawa & Nakashima (2013) and were deposited as AB906671–AB906673.

Complementary strands were assembled using MEGA5 (Tamura *et al.* 2011). To analyze relationships of our isolates to known *Alternaria*-like species, we aligned our sequence data with sequences of seven strains published by Woudenberg *et al.* (2013; TreeBASE study S14312). Alignments were performed using MUSCLE (Edgar 2004) in MEGA5 with default settings, followed by manual adjustments. Aligned positions with gaps or missing data were eliminated using the COMPLETE DELETION option. Phylogenetic trees were constructed using maximum parsimony in MEGA5 based on the Subtree-Pruning-Regrafting algorithm with SEARCH LEVEL 1 and random sequence addition (10 replicates) to obtain the initial tree. Support for branches was gauged with 500 bootstrap (BS) replicates; values >60 % are shown on the resulting tree (Felsenstein 1985). Maximum likelihood analyses including 500 bootstrap replicates were run using RAxML v. 7.2.6 (Stamatakis & Alachiotis 2010) in RAxMLGUI (Silvestro & Michalak 2012), with the GTR model with gamma-distributed rate variation used as the nucleotide substitution model. *Alternaria brassicicola* was used for an outgroup in both analyses based on the result from Lawrence *et al.* (2013). The generated trees were printed with MEGA5.

RESULTS

Morphological descriptions of isolates

Conidia of the three isolated strains incubated on V8 medium are strikingly different from one another with respect to catenation, body length, and width as a consequence of cell hypertrophy, whereas little morphological variation is observed among these characters on inoculated plants (Table 2.3). In contrast, the typical conidial form—narrowly ellipsoid with a blunt-tapered false beak—is always observed both on artificial media and inoculated plants (Figs. 2.1, 2.2).

Table 2.3. Morphological comparison of the study isolates with previous descriptions of *Alternaria cinerariae*.

Isolates	Catenation	Conidial body			False beak (μm)	Total length (μm)	Substrate
		Length × width (μm)	Transverse septa	Longitudinal septa			
Farfugium isolate (MAFF241266)	3–5(–9)	27–295 × 10–54	2–13	0–15 (ca. 3.3)	Up to 160 × 4.5–9	27–320	On V8
	—	20–100 × 9–33	1–9	0–7 (ca. 1.4)	Up to 55 × 5–9	21–133	On *Senecio* lesions
	—	33–114 × 10–36	0–10	0–7 (ca. 1.6)	Up to 70 × 5.5–8	40–183	On *Farfugium* lesions
	—	20–100 × 9–36	2–11	0–7 (ca. 2.3)	Up to 80 × 0–7.5	33–152	On *Gynura* lesions
	2–4(–6)	18–160 × 8–63	1–14	0–13 (ca. 2.7)	Up to 80 × 4.5–9	18–208	On V8
	—	25–83 × 10–40	1–8	0–6 (ca. 0.8)	Up to 50 × 5–10	24–130	On *Senecio* lesions
	—	28–108 × 10–40	1–8	0–6 (ca. 1.1)	Up to 70 × 4.5–7	28–154	On *Farfugium* lesions
	—	20–130 × 10–37	1–10	0–6 (ca. 1.9)	Up to 60 × 4–8	33–166	On *Gynura* lesions
Senecio isolate (MAFF243059)	2–3	30–138 × 9–46	2–12	0–10 (ca. 2.2)	Up to 123 × 5–9	30–240	On V8
	2–3	23–123 × 8–50	1–10	0–9 (ca. 1.7)	Up to 80 × 5–10	33–155	On lesions
	—	28–103 × 10–36	1–9	0–6 (ca. 1.0)	Up to 50 × 6–9.5	28–134	On *Senecio* lesions
	—	30–133 × 10–45	1–12	0–8 (ca. 1.7)	Up to 85 × 5.5–10	35–190	On *Farfugium* lesions
	—	30–93 × 9–30	1–8	0–3 (ca. 1.0)	Up to 53 × 5–9	30–130	On *Gynura* lesions
A. cinerariae (in Enjoji 1931)	Chain	—	(5–11)	0–3	—	77.5–177.5(–240) × 12.5–25.0	On lesions
(in Ellis 1976)	—	50–140 × 15–40	3–10	1	Up to 80 × 6–9	—	—
(in Yu 2001)	2–5	40–100(–140) × 15–40(–50)	5–10	1–2	Up to 90 × 5–9	—	On V8
(in Simmons 2007)	2–5	—	8–11	1–2	—	160–230 × 32–42	On V8
(in Sakoda et al. 2009)	2–3	55–132.5 × 18–37.5	4–9	0–6	Up to 60 × 5–16.3	—	On *Farfugium* lesions
A. cinerariae (as *A. senecionis*) (in Neergaard 1945)	3–4	42–139.5 × 10.5–48	—	—	24–85.5	24.0–139.5 × 10.5–48	On *Senecio* lesions
Alternaria sp. (in Morikawa 2004)	Solitary	49–108 × 10–20	Present	Present	20–44	—	On *Gynura* lesions

—: No descriptions.

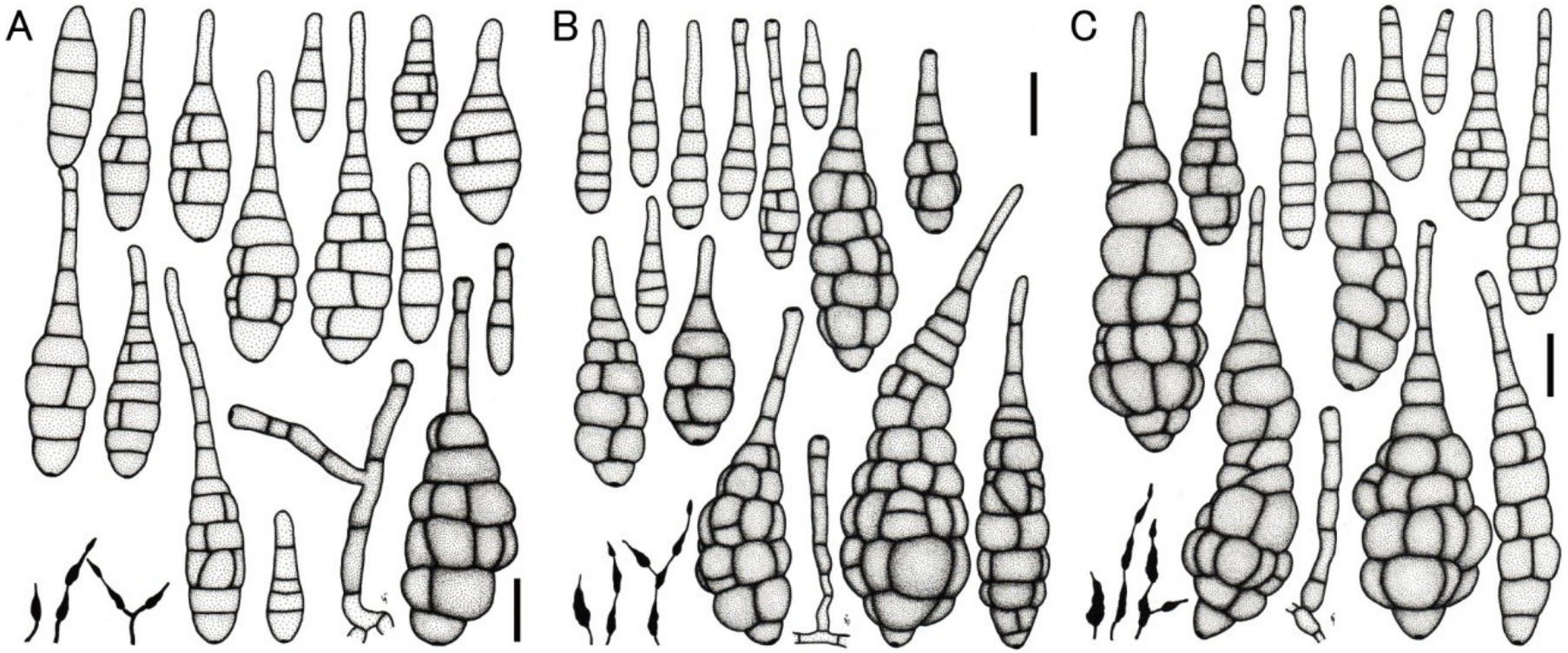

Fig. 2.1. Morphology of conidia and conidiophores and illustrations of sporulation patterns (opaque) on V8 juice agar. **A.** Senecio isolate (MAFF243059). **B.** Farfugium isolate (MAFF241266). **C.** Gynura isolate (MAFF241267). Bars = 25 µm (applies to conidia and conidiophores).

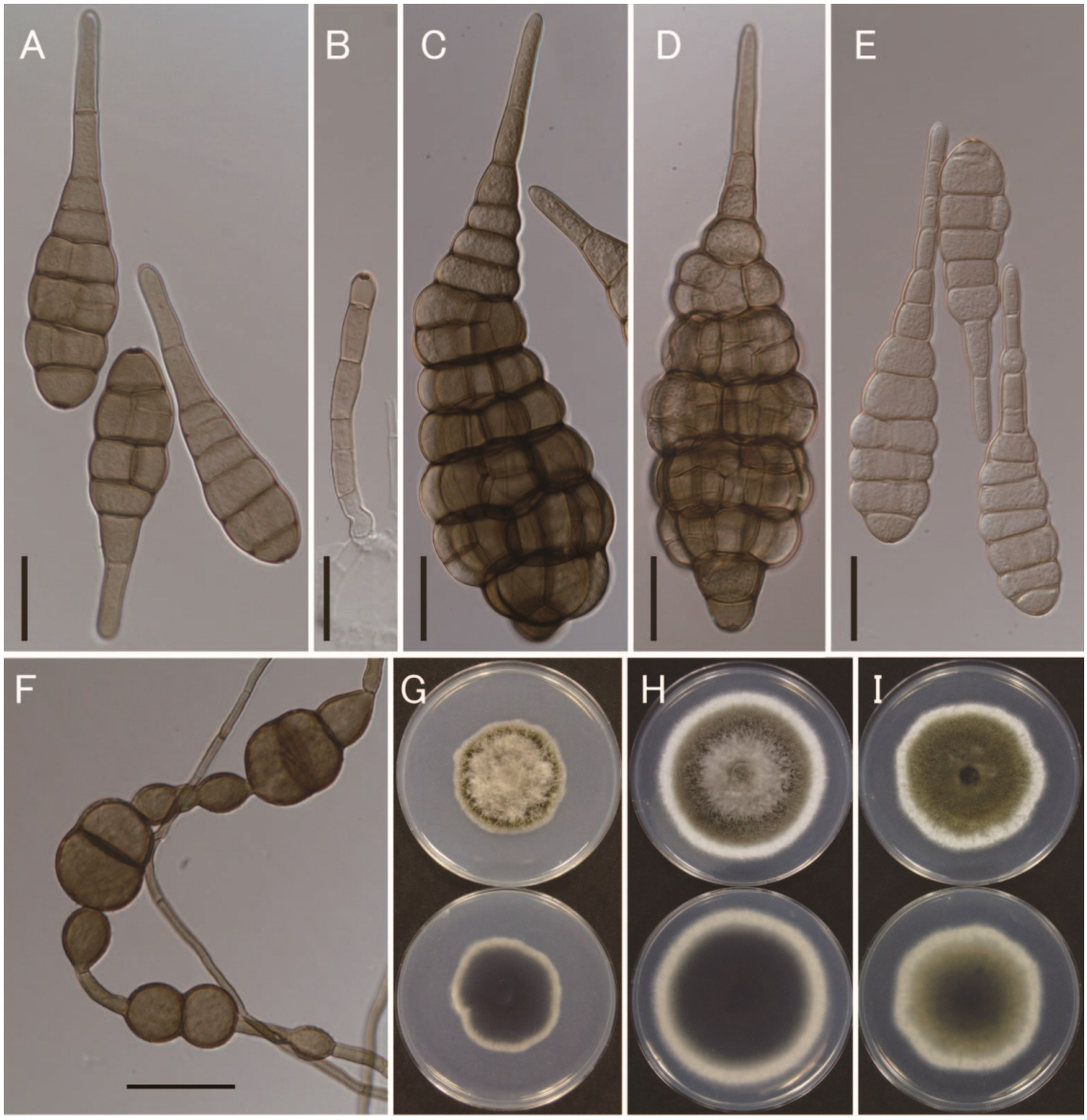

Fig. 2.2. Morphological features of Japanese isolates of *Alternaria cinerariae*. **A.** Typical mature conidia of the Senecio isolate (MAFF 243059) on V8 juice agar. **B.** Conidiophore of the Senecio isolate on V8. **C.** Typical mature (swollen) conidium of the Farfugium isolate (MAFF 241266) on V8. **D.** One of the typical mature conidia of the Gynura isolate (MAFF 241267) on V8. **E.** Terminal conidia in chains of the Gynura isolate. **F.** Chlamydospores of the Gynura isolate produced on potato-carrot agar. **G–I.** Potato-dextrose agar (PDA) colonies of Senecio, Farfugium, and Gynura isolates, respectively (top row = surface, bottom row = reverse). Bars = 25 µm.

Conidial morphology of the Senecio isolate (Figs 2.1A, 2.2A) is consistently identical to that of typical *A. cinerariae* as described in Simmons (2007). Conidia formed on V8 are solitary to 2–3 in a chain, rarely with lateral branches in 5–7 d. Conidia are faintly yellowish-tan to pale brown, long ellipsoid to obclavate, with a blunt-tapered false beak, mostly straight and laterally symmetrical, 30–240 µm in total length, constricted at each transverse segment, and smooth. Conidial bodies are 30–138 × 9–46 µm, with 2–12 transverse septa and 0–10 longitudinal septa. False beaks are unbranched, up to 123 × 5–9 µm, colored, having an inconspicuous border with the conidial body. Chlamydospores (microsclerotia) are absent. Conidiophores are broad, 30–196 × 7.5–9.5 µm, unbranched or often branched (Fig. 2.2B). Colonies are moderate-growing, reaching 31 and 45 mm in diam at 25 °C after 5 and 7 d, respectively; cottony, gray to grayish green, growing aerial mycelia with white margins, black to dark green on the reverse center, rounded at the circumference, without pigment release into the medium (Fig. 2.2G); sporulating sparsely.

Conidia of the Farfugium isolate (Figs 2.1B, 2.2C) on V8 are solitary, predominantly in chains of 3–5(–9), often with one lateral branch in 5–7 d. Conidia are yellowish-tan to brown, long ellipsoid to obclavate, with a blunt-tapered false beak, straight to sometimes curved, 27–320 µm in total length, obviously constricted at each transverse segment, with a smooth surface. Conidial bodies are often excessively swollen, 27–295 × 10–54 µm, with 2–13 transverse septa and 0–15 longitudinal septa. False beaks are unbranched, up to 160 × 4.5–9 µm, colored. Chlamydospores are absent. Conidiophores are broad, 40–105 × 5.5–9.5 µm, unbranched to occasionally branched. Colonies are fast-growing, reaching 53 and 71 mm in diam at 25 °C after 5 and 7 d, respectively, and are rounded in circumference (Fig. 2.2H). Other features of the colony are the same as in the Senecio isolate.

Conidia of the Gynura isolate (Figs. 2.1C, 2.2D, E) on V8 are primarily solitary, frequently in chains of 2–4(–6) with lateral branches at the basal conidium in 5–7 d. Conidia are yellowish-tan to brown, long ellipsoid to obclavate, with a blunt-tapered false beak, straight to sometimes curved, 18–208 µm in total length, obviously constricted at each transverse segment, smooth. Conidial bodies are often excessively swollen, 18–160 × 8–63 µm, with 1–14

transverse septa and 0–13 longitudinal septa. False beaks are unbranched, up to 80 × 4.5–9 µm, colored. Chlamydospores are uncommon on V8, but abundant in aerial mycelia on PCA (Fig. 2.2F). Conidiophores are broad, 25–180 × 6–10 µm, unbranched to occasionally branched. Colonies are fast-growing, reaching 47 and 63 mm in diam at 25 °C after 5 and 7 d, respectively; rounded to sometimes irregular at circumference (Fig. 2.2I). Other colony features are the same as those of the above two isolates.

Materials examined: on *Pericallis cruenta*, Japan, Chiba, Narita, 25 Oct. 2002, by J. Nishikawa (culture MAFF 243059; deposited sequences, *act*: AB906672, *Alt a 1*: AB906671, *gapdh*: AB906670, ITS: AB906673); on *Farfugium japonicum*, Japan, Ibaraki, Tsukuba, Nov. 2008, by T. Sato and Y. Otani (culture MAFF 241266; deposited sequences, *gapdh*: AB862970, *rpb2*: AB862976, *tef1*: AB862982); on *Gynura bicolor*, Japan, Ibaraki, Tsukuba, Nov. 2008, by T. Sato and Y. Otani (culture MAFF 241267; deposited sequences, *gapdh*: AB862971, *rpb2*: AB862977, *tef1*: AB862983).

Characterization of described species

Phylogenetic analysis

PCR amplification of *rpb2* and *tef1* regions of the Senecio isolate MAFF 243059 failed, and thus this isolate was excluded from combined analysis. On the other hand, successfully amplified *gapdh* and additional sequences (*act*, *Alt a 1*, and ITS) in a DDBJ BLASTn search showed 99–100 % similarity to corresponding sequences of Simmons' representative strains including CBS116495 (*gapdh*: KC584109 and AY562413, *act*: JQ671683, *Alt a 1*: AY563308, ITS: KC584190).

The combined *gapdh*, *rpb2*, and *tef1* sequence alignment comprised 1325 characters (*gapdh* = 518, *rpb2* = 614, *tef1* = 193). Phylogenetic analysis of the combined sequence data generated five most parsimonious trees, one of which is shown as Fig. 2.3. Maximum likelihood analysis of the same dataset generated a tree whose groups were also identical to those obtained by maximum parsimony. In the tree shown in Fig. 2.3, the examined isolates are

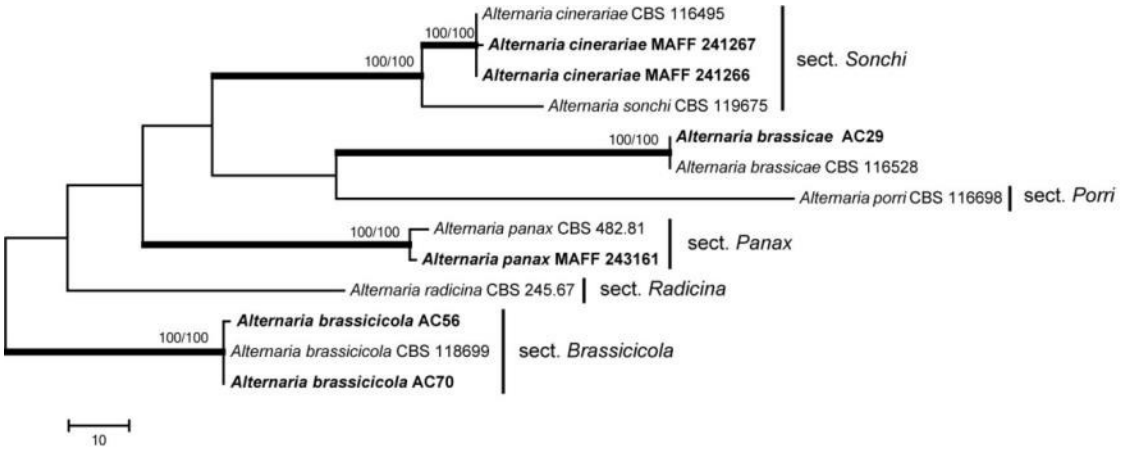

Fig. 2.3. One of five most-parsimonious trees generated from an analysis of *gapdh*, *rpb2*, and *tef1* combined datasets. Parsimony and maximum likelihood bootstrap values > 60 % (500 replications) are shown above branches. Tree length = 382, consistency index = 0.761, retention index = 0.848, and composite index = 0.686. The scale bar indicates the number of nucleotide substitutions. Japanese isolates of *Alternaria* newly sequenced in this study are in boldface.

members of sections circumscribed by Lawrence *et al.* (2013) and Woudenberg *et al.* (2013), all of which are strongly supported. Our two sequenced *A*. *cinerariae* isolates, MAFF 241266 and MAFF 241267, and *A*. *cinerariae* strain CBS 116495 cluster together in a strongly supported (BS = 100 %) monophyletic group. This group in turn is closely related to *A. sonchi* Davis, with which it constitutes a strongly supported (BS = 100 %) clade, congruent with sect. *Sonchi* as emended by Woudenberg *et al.* (2013).

Conversely, the two *A. brassicae* (Berk.) Sacc. strains cluster in a strongly supported (BS = 100 %) monophyletic group sister to sect. *Sonchi*. This result is consistent with the circumscription of Woudenberg *et al.* (2013), who excluded *A. brassicae* from sect. *Sonchi*.

Pathogenicity

According to the results of cross inoculation tests, the isolates, regardless of their original hosts, were pathogenic to inoculated plants of four inoculated *Senecioneae* species—*F. japonicum*, *G.*

bicolor, *J. maritima*, and *P. cruenta*—within 10 dpi (Fig. 2.4A–D). Symptoms first appeared on inoculated leaves of the *Senecioneae* species as brown to black, water-soaked spots; these spots subsequently enlarged and became confluent, similar to those on naturally diseased leaves. On another *Senecioneae* species, *Euryops pectinatus* Cass., positive or negative results were observed depending on inocula: black spots to leaf blight appeared on leaves inoculated with Farfugium and Gynura isolates within 10 dpi, whereas leaves with Senecio isolate barely showed any distinct symptoms (Fig. 2.4E). On *Centaurea cyanus* L., all isolates produced water-soaked black lesions with poor sporulation within 30 dpi (Fig. 2.4F). On leaves of *Callistephus chinensis* (L.) Nees, distinct brown spots appeared and gradually enlarged with rare sporulation by 30 dpi (Fig. 2.4G). On *Cosmos bipinnatus* Cav., numerous spots appeared and manifested as leaf blight with sporulation within 7 dpi (Fig. 2.4H), whereas no distinct symptoms or sporulation were evident on *Cosmos sulphureus* Cav. (Fig. 2.4I). The other inoculated plants—various members of subfamilies *Asteroideae* and *Cichorioideae*—scarcely showed any distinct symptoms (Fig. 2.4J), although spots without sporulation were sometimes observed on inoculated leaves of *Gerbera hybrida* Hort., *Lactuca sativa* L., *Tagetes patula* L., *Taraxacum officinale* F.H. Wigg., and *Zinnia elegans* Jacq. Inoculated leaves of *Cucumis sativa* and *Clarkia amoena* showed no distinct symptoms within 30 dpi (Fig. 2.4K).

DISCUSSION

Conidial swellings (hypertrophy) on aged cultures are a typical characteristic of *A. cinerariae* (Simmons 2007). In this study, however, we frequently observed this feature even on young cultures originating from single conidia formed on *Farfugium* or *Gynura* plants. Moreover, presence or absence of chlamydospores has been considered to be a key character for separating *A. cinerariae* from *A. argyranthemi* E.G. Simmons & C.F. Hill, although the taxonomy of *Alternaria* is primarily based on sporulation patterns (Simmons 2007). Two of the isolates, i.e., those from *Farfugium* and *Gynura*, possessed relatively long chains and often formed

Fig. 2.4. Symptomatology of *Alternaria cinerariae*. **A.** Leaves of *Jacobaea maritima* at 7 d post-inoculation (dpi). **B.** Leaves of *Farfugium japonicum* at 8 dpi. **C.** Leaves of *Gynura bicolor* at 5 dpi. **D.** Leaves of *Pericallis cruenta* at 10 dpi. **E.** Leaves of *Euryops pectinatus* inoculated with Senecio isolate (MAFF 243059; left), Farfugium isolate (MAFF 241266; center), and Gynura isolate (MAFF 241267; right) at 10 dpi. **F.** Leaves of *Centaurea cyanus* at 7 dpi. **G.** Leaves of *Callistephus chinensis* at 7 dpi. **H.** Leaves of *Cosmos bipinnatus* at 7 dpi. **I.** Leaves of *Cosmos sulphureus* at 9 dpi. **J.** Leaves of *Lactuca sativa* at 30 dpi. **K.** Leaves of *Cucumis sativus* at 30 dpi. **L.** Natural symptoms of *G. bicolor*.

chlamydospores (Figs 2.1B, C, 2.2F). Among the isolates identified as *A. cinerariae*, we observed morphological variation in conidial size caused by cell hypertrophy, conidial chain length, and chlamydospore formation. These morphological variations can lead to misidentification of species within sect. *Sonchi*.

The results of the phylogenetic analysis suggest that *A. brassicae* can be excluded from sect. *Sonchi*. Morphologically, conidia of *A. brassicae* are longer and wider than those of *A. cinerariae* and *A. sonchi*, and are usually unconstricted at the septa and characterized by a comparatively lower frequency of longitudinal septa. These differences may be additional evidence supporting the exclusion of *A. brassicae* from sect. *Sonchi*.

The results of inoculation tests indicate that the three Japanese isolates have an identical host range except for their behavior on *E. pectinatus* (Table 2.1). They were strongly pathogenic and produced distinct symptoms with rich sporulation on plants from tribe *Senecioneae* (Fig. 2.4A–E). Although virulence differences among isolates must be considered, two out of three *A. cinerariae* isolates are pathogenic to *E. pectinatus*. Moreover, the susceptible species represent three of the five major subtribes of *Senecioneae* (Pelser *et al.* 2007), which suggest that the *A. cinerariae* isolates are pathogenic to a wide range of *Senecioneae* species. As for tribe *Coreopsideae*, inoculated leaves of *C. bipinnatus* showed severe leaf blight symptoms and sporulation within 7 dpi, whereas those of *C. sulphureus* displayed indistinct symptoms without sporulation after 30 dpi (Fig. 2.4H, I). Taking into account possible overestimation of pathogenicity due to the hypersensitive reaction of the pinnate leaves of *C. bipinnatus*, the genus *Cosmos* is doubtful as a natural host. Inoculated leaves of *C. cyanus* also showed hypersensitive reaction-like spots, as was observed on the other inoculated plants, with poor or no sporulation within 30 dpi (Fig. 2.4F). On the basis of these two positive results, *C. bipinnatus* and *C. cyanus* should be regarded as experimental hosts owing to their hypersensitive reaction. These results suggest that the examined isolates have preferential pathogenicity to *Senecioneae*, and also support our hypothesis that *A. cinerariae* may have host tribe-specific pathogenicity in nature.

This conclusion conflicts with the inoculation test results of Neergaard (1945), who—in

contrast to the findings of our study (Fig. 2.4J)—reported *L. sativa* as a susceptible host of *A. cinerariae* (as *A. senecionis*). Hypersensitive spots are now well known to cause young seedling death; it is thus suspected that the use of young seedlings in his tests may have led to an overestimate of pathogenicity. Because *A. cinerariae* exhibited no pathogenicity to *L. sativa* or most other asteraceous plants, except for those in tribe *Senecioneae*, the host range of the Japanese isolates of *A. cinerariae* might be an important criterion for distinguishing this species from other related species recorded on *L. sativa*, i.e., *A. argyranthemi*, *A. sonchi*, and *A. sudanensis* E.G. Simmons.

Based on the results of the phylogenetic analysis and inoculation tests, the *A. cinerariae* isolates should be treated as a single species, with their morphological and pathological differences representing intraspecific variations. All of our *A. cinerariae* isolates clustered with *A. cinerariae* isolate CBS 116495 into a monophyletic group within sect. *Sonchi* (Fig. 2.3). In addition, *A. cinerariae* was revealed to have pathogenicity not fundamentally restricted as previously believed to the genus *Senecio*. The preferential host-tribe pathogenicity is remarkable because host ranges of many *Alternaria* species are generally limited to a single genus or family, e.g., *A. alstroemeriae* E.G. Simmons & C.F. Hill on *Alstroemeria* (Nishikawa & Nakashima 2013) and *A. brassicae* on plants of family *Brassicaceae* (Bains & Tewari 1987).

Our study has uncovered the first record of *G. bicolor* as a natural host of *A. cinerariae*. Morikawa (2004) also reported a black spot disease on *G. bicolor* caused by *Alternaria* sp. in Toyama Prefecture, Japan. Morphology of its pathogen differed from that of *A. cinerariae* in regard to conidial size and number of conidial catenations, but conidia on his specimens appear to have been immature but typical *A. cinerariae* conidia, possibly reflecting additional *A. cinerariae* intraspecific variation. In our study, natural leaf symptoms on *G. bicolor* were circular to subcircular, often zonate, dark brown to black with a pale brown spot in the center, scattered, enlarged, and confluent, 5–20 mm in diam (Fig. 2.4L).

Our study has also contributed to the characterization of *A. cinerariae* using an integrated species criterion based on phylogenetic relationships, morphological variation, and experimental host range. Our results indicate that host range may be very informative for

determination of *Alternaria* species boundaries.

DISCLOSURE

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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Chapter 3

Morphological and molecular characterization of the strawberry black leaf spot pathogen referred to as the strawberry pathotype of *Alternaria alternata*

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ABSTRACT

The remaining unclarified taxon among the seven known pathotypes of host-selective toxin (HST)-producing *Alternaria alternata*, namely, the strawberry pathotype (the strawberry black leaf spot pathogen), is taxonomically revised and re-described herein. According to our morphological observations, reference isolates of strawberry and Japanese pear pathotypes, which are toxic to leaves of Japanese pear 'Nijisseiki', have conidia that are formed in chains of 3–13, usually without lateral branches, after 7 d incubation on potato-carrot agar. The mean size of the conidia is 27–31 × 11–13 µm. Morphological characteristics of the examined isolates are identical to those of *A*. *gaisen* rather than *A*. *alternata*. A phylogenetic tree obtained by analysis of a combined dataset of ITS, *gapdh*, *rpb2*, *tef1*, *Alt a 1*, and *endoPG* sequences also strongly supports both pathotypes as one species, *A*. *gaisen*. We re-describe the fungus as *A*. *gaisen* Nagano ex Bokura and propose two formae speciales of the species, *A*. *gaisen* f. sp. *fragariae* producing AF-toxin and f. sp. *pyri* producing AK-toxin. The epitype specimen and ex-epitype culture of *A*. *gaisen* are newly designated.

Keywords: *Alternaria alternata* f. sp. *fragariae*, *Alternaria gaisen*, Host-selective toxin, Taxonomy

INTRODUCTION

Black leaf spot of strawberry (*Fragaria* × *ananassa* Duchesne ex Rozier) is an economically important disease (Mass 1998) found thus far only in Japan, New Zealand, Korea, and Italy (Dingley 1970; Watanabe & Umekawa 1977; Cho & Moon 1980; Wada *et al.* 1996; Takahashi *et al.* 1997). The small number of particularly susceptible strawberry cultivars, such as Morioka-16 and Robinson, may be the main reason why occurrences of this disease are geographically limited (Takahashi *et al.* 1990). Typical disease-induced lesions on leaves are circular to irregular, 3–5 mm diam, and reddish brown, sometimes with a yellowish halo (Misawa *et al.* 2012). The causal pathogen has been determined to be *Alternaria alternata* (Fr.) Keissl. producing a host-selective toxin (HST), AF-toxin (Maekawa *et al.* 1984; Nakatsuka *et al.* 1986).

Seven HST-producing *A. alternata* pathotypes are known, namely, the pathogens of *Alternaria* blotch of apple (*Malus domestica* Borkh.) producing AM-toxin, black spot of Japanese pear (*Pyrus pyrifolia* (Burm. f.) Nakai var. *culta* (Makino) Nakai) producing AK-toxin, brown spot of tangerine (*Citrus reticulata* Blanco) producing ACT-toxin, leaf spot of rough lemon (*C. jambhiri* Lush.) producing ACR-toxin, stem canker of tomato (*Solanum lycopersicum* L.) producing AAL-toxin, brown spot of tobacco (*Nicotiana tabacum* L.) producing AT-toxin, and black leaf spot of strawberry producing AF-toxin (Tsuge *et al.* 2013). Mycologists and pathologists have disagreed in regards to the taxonomy of these pathogens. Simmons (1999) recognized each pathogen (except for the strawberry pathogen) as a distinct morphospecies distinguishable from *A. alternata* and each other on the basis of conidial size and sporulation patterns: *A. mali* Roberts on apple, *A. gaisen* Nagano on Japanese pear, *A. toxicogenica* E.G. Simmons on tangerine, *A. limoniasperae* E.G. Simmons on rough lemon, *A. arborescens* E.G. Simmons on tomato, and *A. longipes* (Ellis & Everh.) E.W. Mason on tobacco. In contrast, some Japanese pathologists favoring the pathotype concept have considered the HST-producing *Alternaria* to be intraspecific variations of *A. alternata*, as they are nearly morphologically undifferentiated from one another (Nishimura 1980; Nishimura & Kohmoto, 1983) and most phylogenetic studies have found them to be indistinguishable from *A. alternata* (Kusaba &

Tsuge, 1994, 1995, 1997; Peever *et al.* 2004, 2005; Andrew *et al.* 2009; Rotondo *et al.* 2012; Armitage *et al.* 2015). However, *A*. *arborescens*, *A*. *gaisen*, and *A*. *longipes* are recognizable as distinct taxa, and several studies based on random amplified polymorphic DNA fragment pattern analysis, high performance liquid chromatography analysis of secondary metabolites, and multi-locus phylogeny have not supported such a pathotype designation (Roberts *et al.* 2000; Andersen *et al.* 2001; Peever *et al.* 2004; Armitage *et al.* 2015; Ozkilinc *et al.* 2018).

In a recent study based on genome and transcriptome comparisons and phylogenetic analyses of genome sequences generated by next-generation sequencing, Woudenberg *et al.* (2015) concluded that the seven pathotypes should be classified into three distinct species (*A*. *gaisen*, *A*. *longipes*, and an *A*. *arborescens* species complex) and three formae speciales of *A*. *alternata* (with one, f. sp. *citri*, including two pathotypes). In regards to the *A*. *alternata*-like pathogen on strawberry, *A*. *alternata* f. sp. *fragariae* in Woudenberg *et al.* (2015), however, we are doubtful that the examined isolate was the AF-toxin producer because Misawa *et al.* (2012) pointed out that the pathogen has morphological differences from *A*. *alternata*. Consequently, the taxonomy of the strawberry pathotype is still unclear.

In this study, our goal was to reclassify the HST-producing *A*. *alternata*-like species pathogenic to strawberry on the basis of morphological and phylogenetic analyses using reliable strains. We also aimed to provide a taxonomically correct name for the pathogen.

MATERIALS AND METHODS

Fungal isolates

Seven *A*. *alternata*-like isolates (MAFF 731001–731007) deposited at the Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Japan, were used in this study. These isolates were the reference isolates of the strawberry pathotype of *A*. *alternata* described in Watanabe & Umekawa (1977) and Watanabe *et al.* (1978). We additionally examined one isolate of *Alternaria* sp. (MAFF 242310) from black leaf spot symptoms of

strawberry and producing AF-toxin (Misawa *et al.* 2012; Yamamoto *et al.* 2012), three isolates (MUCC 2151–2153) of the causal pathogen of black spot of Japanese pear, and three isolates morphologically identified as *A. alternata* from various hosts, to reveal their morphological differences and phylogenetic relationships (Table 3.1).

Morphological observations

To obtain diagnostic conidia and conidial catenation, growing colonies incubated at 25 °C in the dark for 7 d on potato-carrot agar (PCA; Simmons 2007) were scratched with a flame-sterilized needle and the aerial mycelia were removed to observe sporulation more easily. After 12–24 h of pre-incubation in unsealed Petri dishes at 25 °C under blacklight blue fluorescent lamps to induce sporulation (Nishikawa & Nakashima 2013), the plates were incubated at 20 °C in the dark, and sporulation patterns were observed for 7 d afterwards. Conidia formed on the medium 7 d after incubation were mounted with Shear's mounting fluid [300 mL aqueous potassium acetate (2 %), 120 mL glycerin, and 180 mL ethanol (95 %)] for microscopic observation. The morphology of 100 conidia was examined at 400× magnification.

Assay for toxicity

Detached young leaves of Japanese pear 'Nijisseiki' susceptible to both AK- and AF-toxins produced by *A. alternata*-like species on Japanese pear and strawberry (Hayashi *et al.* 1992) were prepared for a susceptibility assay according to Abe *et al.* (2010). Young leaves detached from growing shoots were rinsed with distilled water. The leaves were dried on sterilized filter paper and then placed upside down on a moistened paper towel in a plastic chamber. Conidia formed on V8 juice agar medium (Simmons 2007) were washed with sterilized distilled water containing 0.02 % polyoxyethylene (20) sorbitan monolaurate (Wako Pure Chemicals, Osaka) and rubbed with a sterilized plastic spreader. Detached conidia from conidiophores were harvested and used as inocula. Concentrations of conidial suspensions of each isolate were adjusted to a range from 1×10^5 to 5×10^5 conidia/mL. Conidial suspensions of 20 µl each were dropped on the leaves, with the inoculated leaves then maintained under moist conditions at

Table 3.1. Isolates phylogenetically analyzed in this study and their DDBJ accession numbers.

Fungal name and isolate number[1, 2]	Country, host plant	DDBJ/GenBank/EMBL accession numbers[3]					
		ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*
Alternaria alstroemeriae							
CBS 118809^{T}	Australia, *Alstroemeria* sp.	KP124297	KP124154	KP125072	KP124765	–	KP123994
MAFF 241374	Japan, *Alstroemeria* sp.	AB678214	AB744034	**LC275050**	**LC275231**	AB744031	**LC276240**
A. alternata							
MUCC 1610	Japan, *Impatiens hawkeri*	**LC269968**	**LC270135**	**LC275052**	**LC275233**	**LC276230**	**LC276242**
MUCC 1611	Japan, *Antirrhinum majus*	–	**LC270134**	**LC275051**	**LC275232**	**LC276229**	**LC276241**
MUCC 1616	Japan, *Pelargonium hortorum*	**LC269969**	**LC270136**	**LC275053**	**LC275234**	**LC276231**	**LC276243**
CBS 916.96^{T}	India, *Arachis hypogaea*	AF347031	AY278808	KC584634	KC584375	AY563301	JQ811978
CBS 121348 (= *A. platycodonis*^{T})	China, *Platycodon grandiflorus*	KP124367	KP124219	KP125144	KP124836	KP123915	KP124070
A. alternata f. sp. *citri* pathotype rough lemon (= *A. alternata* rough lemon pathotype)							
CBS 102595 (= *A. limoniasperae*^{T})	USA, *Citrus jambhiri*	FJ266476	AY562411	KC584666	KC584408	AY563306	KP124029
A. alternata f. sp. *citri* pathotype tangerine (= *A. alternata* tangerine pathotype)							
CBS 102600 (= *A. toxicogenica*^{T})	USA, *Citrus reticulata*	KP124331	KP124186	KP125107	KP124799	KP123880	KP124033
A. alternata f. sp. *mali* (= *A. alternata* apple pathotype)							
CBS 106.24 (= *A. mali*^{T})	USA, *Malus sylvestris*	KP124298	KP124155	KP125073	KP124766	KP123847	AY295020
A. alternantherae							
CBS 124392	China, *Solanum melongena*	KC584179	KC584096	KC584633	KC584374	KP123846	–
A. arborescens (= *A. alternata* tomato pathotype)							
CBS 102605^{T}	USA, *Solanum lycopersicum*	AF347033	AY278810	KC584636	KC584377	AY563303	AY295028
A. betae-kenyensis							
CBS 118810^{T}	Kenya, *Beta vulgaris* var. *cicla*	KP124419	KP124270	KP125197	KP124888	KP123966	KP124123
A. burnsii							
CBS 107.38	India, *Cuminum cyminum*	KP124420	JQ646305	KP125198	KP124889	KP123967	KP124124

Table 3.1. (Continued).

Fungal name and isolate number[1, 2]	Country, host plant	DDBJ/GenBank/EMBL accession numbers[3]					
		ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*
A. eichhorniae							
CBS 489.92[T]	India, *Eichhornia crassipes*	KC146356	KP124276	KP125204	KP124895	KP123973	KP124130
A. gaisen f. sp. *fragariae* (= *A. alternata* strawberry pathotype)							
MAFF 731001	Japan, *Fragaria* × *ananassa*	**LC164854**	**LC169125**	**LC167148**	**LC169131**	**LC276235**	**LC276246**
MAFF 731002	Japan, *Fragaria* × *ananassa*	**LC164853**	**LC169126**	**LC167149**	**LC169132**	**LC276236**	–
MAFF 731003	Japan, *Fragaria* × *ananassa*	**LC164852**	**LC169127**	**LC167150**	**LC169133**	**LC167085**	**LC276247**
MAFF 731004	Japan, *Fragaria* × *ananassa*	**LC164851**	**LC270140**	**LC167151**	**LC275238**	–	**LC276248**
MAFF 731005	Japan, *Fragaria* × *ananassa*	**LC164850**	**LC169128**	**LC167152**	**LC169134**	**LC167086**	**LC276249**
MAFF 731006	Japan, *Fragaria* × *ananassa*	**LC164849**	**LC169129**	**LC275057**	**LC169135**	**LC167087**	**LC276250**
MAFF 731007	Japan, *Fragaria* × *ananassa*	**LC164848**	**LC169130**	**LC275058**	**LC169136**	**LC167088**	**LC276251**
MAFF 242310	Japan, *Fragaria* × *ananassa*	**LC269973**	**LC270141**	**LC275059**	**LC275239**	**LC276237**	**LC276252**
A. gaisen f. sp. *pyri* (= *A. alternata* Japanese pear pathotype)							
CBS 118488[T]	Japan, *Pyrus pyrifolia* var. *culta*	KP124427	KP124278	KP125206	KP124897	KP123975	KP124132
CBS 632.93[R]	Japan, *Pyrus pyrifolia* var. *culta*	KC584197	KC584116	KC584658	KC584399	KP123974	AY295033
MUCC 2151	Japan, *Pyrus pyrifolia* var. *culta*	**LC269970**	**LC270137**	**LC275054**	**LC275235**	**LC276232**	–
MUCC 2152	Japan, *Pyrus pyrifolia* var. *culta*	**LC269971**	**LC270138**	**LC275055**	**LC275236**	**LC276233**	**LC276244**
MUCC 2153	Japan, *Pyrus pyrifolia* var. *culta*	**LC269972**	**LC270139**	**LC275056**	**LC275237**	**LC276234**	**LC276245**
A. gossypina							
CBS 104.32[T]	Zimbabwe, *Gossypium* sp.	KP124430	JQ646312	KP125209	KP124900	JQ646395	KP124135
A. iridiaustralis							
CBS 118486[T]	Australia, *Iris* sp.	KP124435	KP124284	KP125214	KP124905	KP123981	KP124140
A. iridicola							
MUCC 2148	Japan, *Iris japonica*	**LC269974**	**LC270142**	**LC275060**	**LC275240**	**LC276238**	**LC276253**
MUCC 2149	Japan, *Iris japonica*	**LC269975**	**LC270143**	**LC275061**	**LC275241**	**LC276239**	**LC276254**
A. jacinthicola							

Table 3.1. (Continued).

Fungal name and isolate number[1, 2]	Country, host plant	DDBJ/GenBank/EMBL accession numbers[3]					
		ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*
CBS 133751^{T}	Mali, *Eichhornia crassipes*	KP124438	KP124287	KP125217	KP124908	KP123984	KP124143
A. longipes (= *A. alternata* tobacco pathotype)							
CBS 540.94^{R}	USA, *Nicotiana tabacum*	AY278835	AY278811	KC584667	KC584409	AY563304	KP124147
CBS 121332^{R}	USA, *Nicotiana tabacum*	KP124443	KP124292	KP125227	KP124913	KP123989	KP124149
CBS 121333^{R}	USA, *Nicotiana tabacum*	KP124444	KP124293	KP125223	KP124914	KP123990	KP124150
A. tomato							
CBS 103.30	Unknown, *Solanum lycopersicum*	KP124445	KP124294	KP125224	KP124915	KP123991	KP124151
CBS 114.35	Unknown, *Solanum lycopersicum*	KP124446	KP124295	KP125225	KP124916	KP123992	KP124152

[1] CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; MAFF: Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Japan; MUCC (Japan): Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Japan.

[2] T: ex-type and ex-epitype strain; R: representative strain of Simmons (2007); fungal names between parentheses are their former names or their names under the pathotype concept (Nishimura 1980).

[3] Boldface accession numbers refer to sequences generated in this study.

20 °C in the dark. After 7 d incubation, we looked for necrotic lesions around the applied points. Comparable isolates belonging to *Alternaria* sect. *Alternaria* D.P. Lawr. *et al.*, including *A. alstroemeriae* E.G. Simmons & C.F. Hill (MAFF 241374), *A. iridicola* (Ellis & Everh.) J.A. Elliot (MUCC 2149), and *A. alternata* (MUCC 1610, MUCC 1611, and MUCC 1616), were also used. The inoculation tests were repeated three times.

DNA extraction and phylogenetic analyses

Total genomic DNA was extracted from mycelial discs using an UltraClean Microbial DNA isolation kit (MoBio Laboratories, Carlsbad) according to the manufacturer's instructions. PCR amplification and sequencing was carried out for the following DNA regions: the rDNA internal transcribed spacer (ITS) region as described in Nishikawa & Nakashima (2013); glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), RNA polymerase second largest subunit (*rpb2*), and translation elongation factor 1-alpha (*tef1*) genes as described in Nishikawa & Nakashima (2015); and Alternaria major allergen (*Alt a 1*) and endopolygalacturonase (*endoPG*) genes using primers Alt-for and Alt-rev (Hong *et al.* 2005) for *Alt a 1* and PG3 and PG2b (Andrew *et al.* 2009) for *endoPG* as described in Woudenberg *et al.* (2014) and Woudenberg *et al.* (2015). PCR products were labeled using a BigDye Terminator v.3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems, Carlsbad) on a T100 thermal cycler and then sequenced in both directions on a 3730x1 Genetic Analyzer (Applied Biosystems) at the Mie University Advanced Science Research Promotion Center, Japan. All newly determined sequences were deposited in the DNA Data Bank of Japan (DDBJ) (Table 3.1).

Complementary strands of the generated sequences and those of 20 related species within sect. *Alternaria* retrieved from DDBJ were assembled and concatenated in MEGA version 7 (Kumar *et al.* 2016) and aligned using MAFFT v. 7 (Katoh *et al.* 2017; http://mafft.cbrc.jp/alignment/server/index.html). Sequence alignments were deposited in TreeBASE under accession number S21736. Phylogenetic trees were constructed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) based on a combined dataset of ITS, *gapdh*, *rpb2*, *tef1*, *Alt a 1*, and *endoPG* sequences. MP analyses were

performed in PAUP* v. 4.0b10 (Swofford 2003), with heuristic searches consisting of 1,000 random sequence additions and tree-bisection-reconnection branch swapping. Alignment gaps were treated as fifth bases and all characters were unordered and of equal weight. The robustness of the obtained trees was evaluated by 1,000 bootstrap (BS) replications (Felsenstein 1985). Tree scores, including tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC), were also calculated. ML analyses were performed in RAxML BlackBox (Stamatakis, Hoover, & Rougemont, 2008) using the GAMMA model and 100 rapid bootstrap replicates. BI analyses were performed in MrBayes 3.2.6 (Ronquist *et al.* 2012) to estimate the posterior probabilities (PPs) of tree topologies based on Metropolis-coupled Markov chain Monte Carlo searches (MCMCMC). A nucleotide substitution model GTR+I+G was selected by Kakusan4 software (Tanabe 2011). A MCMC algorithm of four chains was started in parallel from a random tree topology with the heating parameter set at 0.1. The MCMC analysis lasted until 1,000,000 generations. Trees were sampled and saved every 1,000 generations. The first 25 % of saved trees were discarded as the "burn-in" phase and PPs determined from the remaining trees. Sequences of *A. alternantherae* (CBS 124392) from a previous study (Woudenberg *et al.* 2015) were used as the outgroup. The generated trees were printed with FigTree v. 1.4.2 (Institute of Evolutionary Biology, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree).

RESULTS

Conidial morphology and catenation

Sporulation patterns and conidial characteristics of the examined isolates originating from strawberry and Japanese pear (MAFF 731001–731007, MAFF 242310, and MUCC 2151–2153) were identical to those of *A. gaisen* described in Simmons (1993, 1999, 2007) except for two unstable and sparsely sporulating isolates (MAFF 731004 and MUCC 2153) (Table 3.1).

Conidial formation on PCA was in chains of 3–13. The chains usually remained unbranched within 7 d incubation, or occasionally arose as short lateral branches from a primary chain. Conidial bodies were ovoid to ellipsoid, sometimes obclavate, pale to olive brown; their mean size ranged from 27–31 × 11–13 µm, with up to 8 transverse septa and 0–2 longitudinal septa in each transverse segment, often constricted at near the median transverse septum, and producing 1–2-celled secondary conidiophores at the conidial apex (Fig. 3.1). Conidial morphology of the two unstable isolates (MAFF 731004 and MUCC 2153) was similar to that of *A*. *alternata* rather than *A*. *gaisen*. In contrast, the conidial morphology of the three *A*. *alternata* isolates originating from various hosts (MUCC 1610, MUCC 1611, and MUCC 1616) was almost identical to that of *A*. *alternata* described in Simmons (2007) (Table 3.1). Although the conidia of MUCC 1610 and MUCC 1616 on PCA displayed larger and meager catenation, respectively, those of *A*. *alternata* isolates were mostly smaller and mass-formed in chains with complex lateral branches. According to our observations of conidial morphology and sporulation patterns of the two species, the examined isolates identified as *A*. *gaisen* were morphologically distinguishable from *A*. *alternata* as described in Simmons & Roberts (1993) and Simmons (1999).

Toxicity to Japanese pear 'Nijisseiki' leaves

Necrosis appeared on Japanese pear leaves inoculated with all the isolates identified as *A*. *gaisen*. Each leaf inoculated with one of the seven isolates obtained from diseased strawberry, MAFF 731001–731007, exhibited necrosis at 3 d post-inoculation (dpi) (Fig. 3.2A). The three Japanese pear isolates (MUCC 2151–2153) and MAFF 242310 also caused necrosis, whereas the other examined isolates, including *A*. *alstroemeriae* (MAFF 241374), *A*. *iridicola* (MUCC2149), and *A*. *alternata* (MUCC 1611), produced no symptoms even 14 dpi (Fig. 3.2B). Two additional *A*. *alternata* isolates (MUCC 1610 and MUCC 1616) similarly triggered no symptoms (data not shown).

Table 3.1. Comparative morphology of the strawberry black leaf spot pathogen and related species on potato-carrot agar.

Species and Isolates[1]	Conidial chain	Lateral branch	Conidial size (µm) and number of septa			Toxicity[2]	References
			Length × width (mean values with 95 % confidence interval)	Transverse septa	Longitudinal septa		
Alternaria sp. (= *A. alternata* strawberry pathotype)							
MAFF 242310	4–9	uncommon	12–49 × 6–16 (30 ± 2 × 12 ± 0.4)	0–8	0–6	+	this study
MAFF 731001	6–9	uncommon	16–53 × 8–18 (31 ± 1 × 12 ± 0.4)	2–8	0–4	+	this study
MAFF 731002	3–10	uncommon	16–50 × 7–17 (28 ± 1 × 12 ± 0.4)	2–7	0–5	+	this study
MAFF 731003	6–11	uncommon	15–56 × 7–18 (28 ± 2 × 12 ± 0.5)	2–7	0–4	+	this study
MAFF 731004*	7–16	common	11–53 × 6–16 (25 ± 2 × 10 ± 0.4)	1–6	0–4	+	this study
MAFF 731005	4–11	uncommon	15–53 × 7–17 (28 ± 2 × 12 ± 0.4)	2–8	0–5	+	this study
MAFF 731006	6–10	uncommon	15–67 × 8–18 (29 ± 2 × 13 ± 0.4)	1–7	0–4	+	this study
MAFF 731007	4–13	uncommon	10–47 × 6–16 (27 ± 2 × 11 ± 0.4)	1–8	0–4	+	this study
A. gaisen (= *A. alternata* Japanese pear pathotype)	3–9	unbranched	30–45(–55) × 13–15(–18)	5–8	0–1(–2) in each segment		Simmons 2007
MUCC 2151	6–12	uncommon	15–45 × 6–15 (28 ± 1 × 11 ± 0.3)	2–5	0–4	+	this study
MUCC 2152	5–12	uncommon	15–49 × 8–18 (29 ± 1 × 13 ± 0.4)	2–8	0–5	+	this study
MUCC 2153*	15–23	common	12–42 × 6–13 (22 ± 1 × 9 ± 0.3)	1–7	0–3	+	this study

Table 3.1. (Continued).

Species and Isolates[1]	Conidial chain	Lateral branch	Conidial size (µm) and number of septa			Toxicity[2]	References
			Length × width (mean values with 95 % confidence interval)	Transverse septa	Longitudinal septa		
A. alternata	15–20	multiple-branched	7–25(–40) × 5–12	1–7	very few		Simmons 2007
MUCC 1610	12–20	common	13–50 × 7–16 (28 ± 1 × 12 ± 0.3)	1–7	0–5	-	this study
MUCC 1611	19–22	common	13–41 × 8–18 (23 ± 1 × 12 ± 0.4)	2–6	0–4	-	this study
MUCC 1616	10–14	uncommon	11–36 × 7–15 (23 ± 1 × 11 ± 0.3)	1–6	0–3	-	this study

[1] MAFF: Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Japan; MUCC (Japan): Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Japan.

[2] Toxicity to Japanese pear 'Nijisseiki' leaves based on inoculation testing (see Fig. 3.2). +: toxic, -: non-toxic.

[*] Unstable strains; these two examined isolates (MAFF 731004 and MUCC 2153) produced almost no conidia.

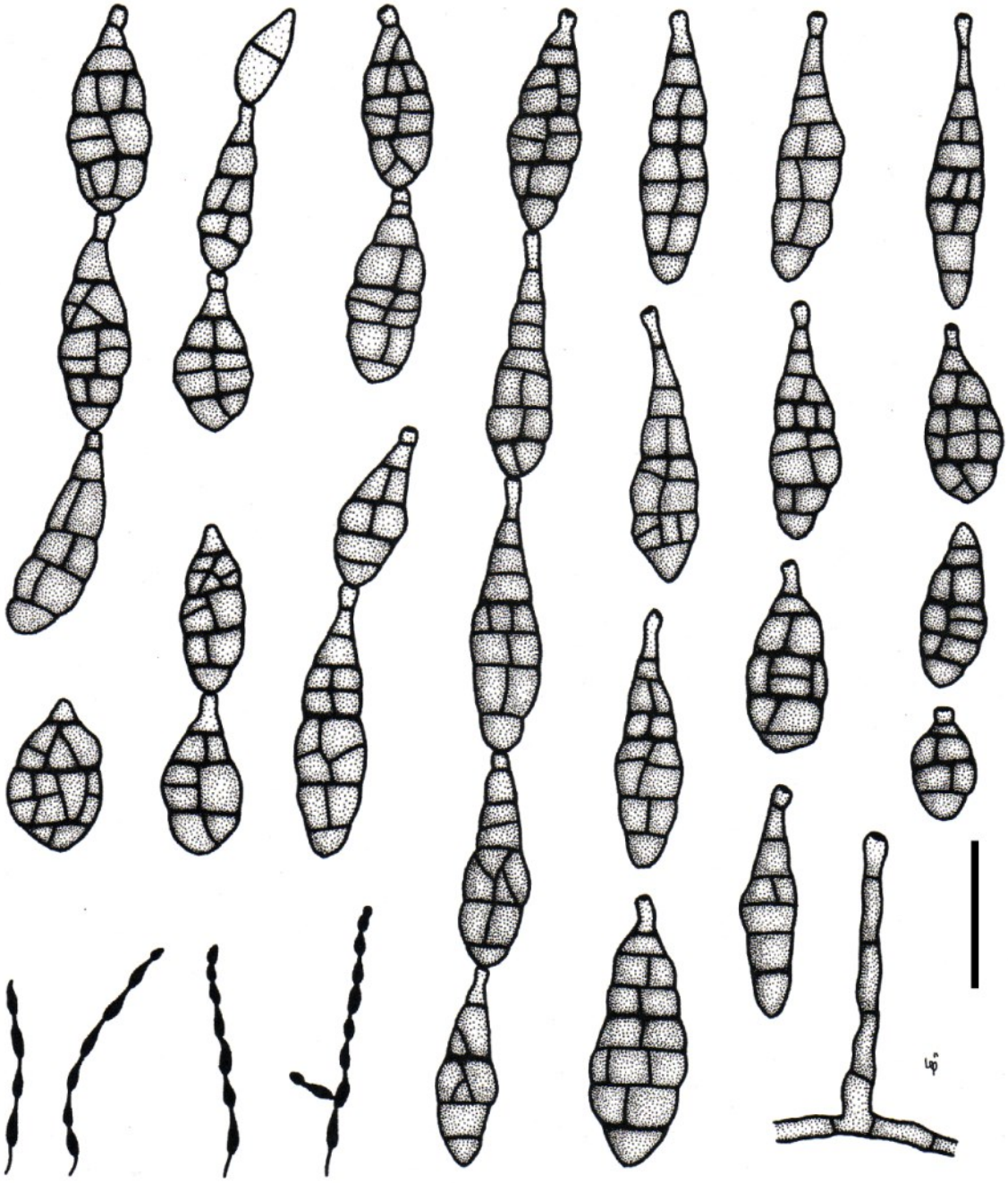

Fig. 3.1. Morphological illustrations of *Alternaria gaisen* f. sp. *fragariae* (MAFF 242310) on potato-carrot agar.

Bar = 25 µm (not applicable to the illustrated sporulation patterns).

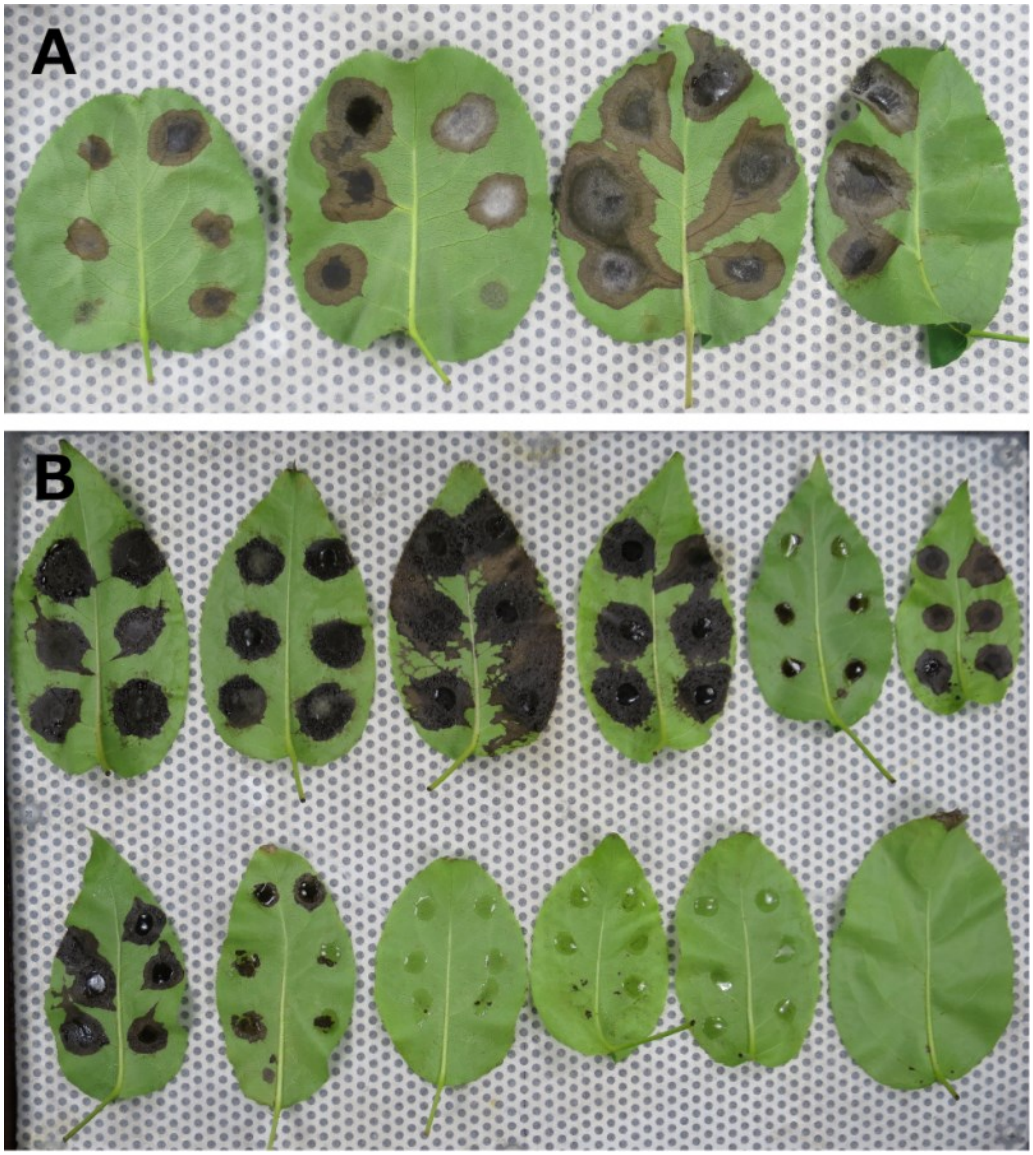

Fig. 3.2. Toxicity of isolates examined in this study to susceptible Japanese pear leaves. **A.** Results using the seven isolates identified as strawberry black leaf spot pathogen at 6 d post-inoculation. Three drops of each inoculum were applied to the left or right side of a leaf. Isolates inoculated on each leaf half, from left to right, are MAFF 731001, MAFF 731002, MAFF 731003, MAFF 731004, MAFF 731005, MAFF 731006, MAFF 731007, and distilled water (negative control). **B.** Results of an inoculation test using isolates from this study at 8 d post-inoculation. Isolates inoculated on each leaf from left to right are as follows: (upper row) MUCC 2151, MUCC 2152, MUCC 2153, MAFF 242310, MAFF 731001, and MAFF 731002; (lower row) MAFF 731005, MAFF 731006, MAFF 241374 (*Alternaria alstroemeriae*), MUCC 2149 (*A. iridicola*), MUCC 1611 (*A. alternata*), and distilled water (negative control).

Phylogenetic analysis

The combined aligned dataset of ITS, *gapdh*, *rpb2*, *tef1*, *Alt a 1*, and *endoPG* sequences had a total length of 3,037 base pairs (ITS = 528, *gapdh* = 582, *rpb2* = 757, *tef1* = 243, *Alt a 1* = 476, and *endoPG* = 451). The topologies of the resulting trees from MP, ML, and BI analyses were congruent with one another. One of the MP trees (TL = 549, CI = 0.834, RI = 0.885, and RC = 0.738) is shown in Fig. 3.3. Although *A*. *alternata* and *A*. *arborescens* were not differentiated from one another, the other 10 species defined by Woudenberg *et al.* (2015) and *A*. *iridicola* within sect. *Alternaria* were well resolved and consistent with current species boundaries. All of our examined isolates (including the former strawberry pathotype and Japanese pear pathotype of *A*. *alternata*), which were morphologically identified as *A*. *gaisen*, and the ex-epitype *A*. *gaisen* strain CBS 118488, clustered together in a strongly supported (MP BS/ML BS/BI PP = 99 %/100 %/1.0) but largely unresolved clade that was sister to *A*. *iridicola*. In terms of the remaining pathotypes, *A*. *arborescens* (tomato pathotype) and two formae speciales of *A*. *alternata*, f. sp. *citri* (rough lemon and tangerine pathotypes) and f. sp. *mali* (apple pathotype), were included in a well-supported clade that was distinct from *A*. *longipes* (tobacco pathotype). The placement of these taxa is consistent with the species delimitations proposed by Woudenberg *et al.* (2015).

Taxonomy

On the basis of conidial morphology and the results of our phylogenetic analysis, the causal pathogen of strawberry black leaf spot is taxonomically defined and described as follows.

Alternaria gaisen Nagano ex Bokura, *J*. *Pl*. *Protect*. (*Tokyo*) **11**: 490. 1924. [MB 823578].

≡ *Alternaria gaisen* Nagano, *J*. *Jpn*. *Hort*. *Soc*. (*Nihon Engei Zasshi*) **32**(3): 16, 1920, nom. inval. (provisional name; ICN Art. 36.1). [MB 542199].

≡ *Alternaria gaisen* Nagano, in Hara, *Jitsuyo Sakumotsu Byorigaku*: 263, 1925.

≡ *Alternaria gaisen* Nagano ex Hara, *Sakumotsu Byorigaku*, Edn 4: 263, 1928, in Woudenberg *et al.*, *Stud*. *Mycol*. **82**: 15, 2015, nom. superfl. [MB 252306].

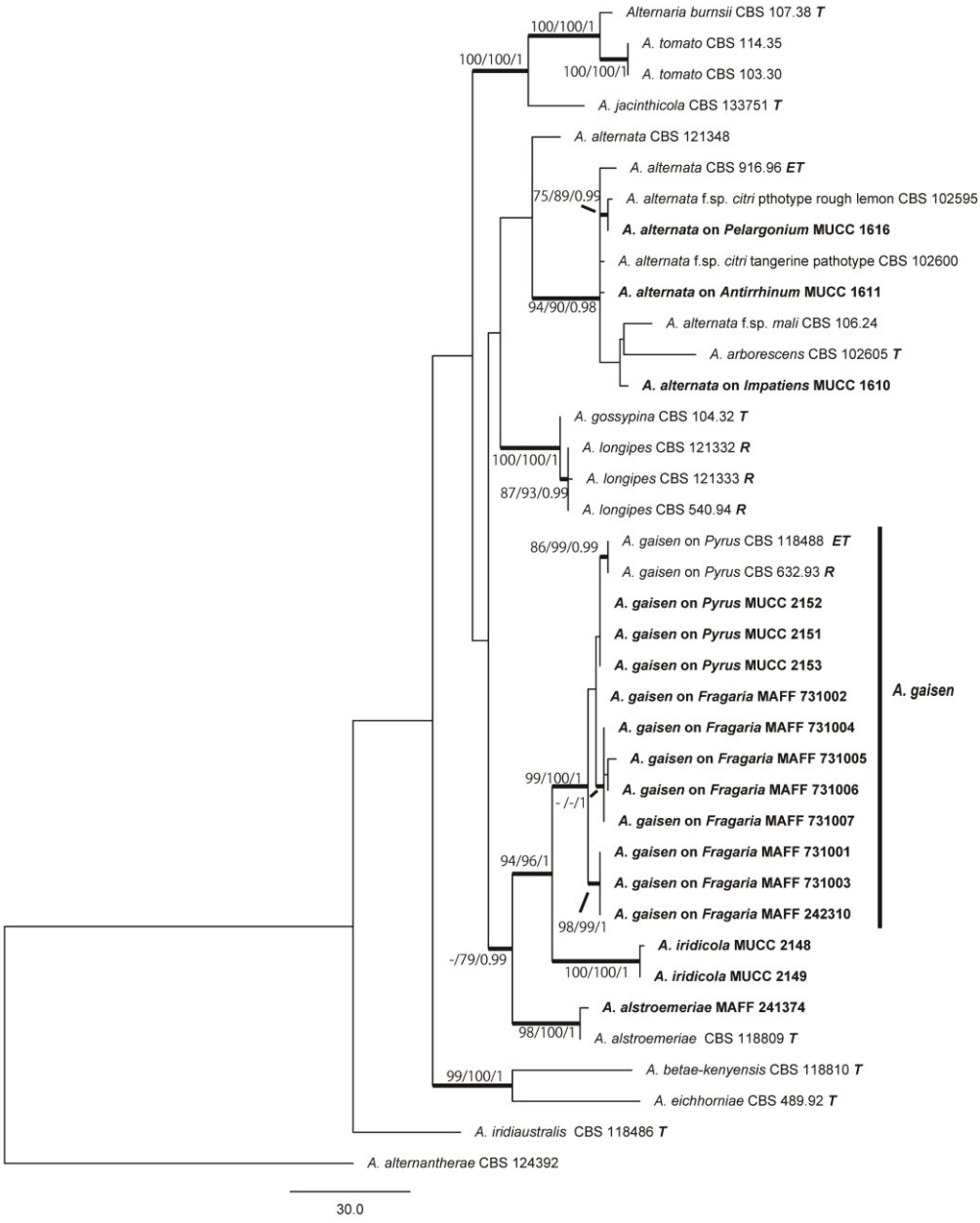

Fig. 3.3. Phylogenetic tree of sect. *Alternaria* generated from analysis of an ITS, *gapdh*, *rpb2*, *tef1*, *Alt a 1*, and *endoPG* combined dataset. Parsimony (MP) and RAxML maximum likelihood (ML) bootstrap values > 60 % and Bayesian posterior probabilities (PP) > 0.98 are shown above branches (MP/ML/PP). Tree length = 549, consistency index = 0.834, retention index = 0.885, and composite index = 0.738. The scale bar indicates the number of nucleotide substitutions. Isolates sequenced in this study are indicated in bold. Status of reference isolates were indicated in bold and italic; T: ex-type, ET: ex-epitype, R: representative strain assigned by Simmons (2007).

= *Alternaria kikuchiana* S. Tanaka, *Mem*. *Coll*. *Agric*. *Kyoto Imp*. *Univ*. **28** (*Phytopathol*. *Ser*. **6**): 27, 1933. [MB 268619].

Conidial formation on PCA (MAFF 731001) in chains of 6–9, commonly without lateral branches. Primary conidiophores macronematous, solitary, emerging from aerial or creeping hyphae, subcylindrical, unbranched, straight or geniculate, 16–86 × 3–5 µm; conidiogenous cells terminal and intercalary, proliferating sympodially, with pores for tretic sporulation. Conidia chained acropetally, ovoid to ellipsoid or obclavate, pale to olive brown, smooth to conspicuously verruculous; conidial bodies 16–53 × 8–18 µm, 31 × 12 µm on average, 2–8 transverse and 0–4 longitudinal septate, slightly constricted at the median and some transverse septa; secondary conidiophores at the apical end of conidia short, mostly single-celled, unbranched, with a pore for tretic sporulation.

Type: **Japan**, Nara Prefecture, on leaves of *P*. *pyrifolia* var. *culta* 'Nijisseiki' (details unknown; not preserved).

Lectotype: Nagano K., *J*. *Jpn*. *Hort*. *Soc*. (*Nihon Engei Zasshi*) **32**(3): 17, Figures (iconotype, selected by Simmons, 2007). [MBT 134588] [Note: Simmons (2007) wrongly cited as "*J*. *Jap*. *Soc*. *Hort*. *Sci*. (*Nippon Engeikai Zasshi)*". The correct title of the journal is cited here]

Epitype (**designated here**): **Japan**, Tottori Prefecture, on *P*. *pyrifolia* var. *culta*, Jul 1990, by E.G. Simmons, dried culture specimen CBS H-22842, living culture CBS 118488 = EGS 90.0391 (representative isolate proposed by Simmons, 2007). [MBT 379199]

Isolates examined: **Japan**, Iwate Prefecture, Morioka, on *Fragaria* × *ananassa* 'Morioka-16', 1975, by Y. Watanabe, M-11 (MAFF 731001), M-14 (MAFF 731002), M-15 (MAFF 731003), M-17 (MAFF 731004), M-20 (MAFF 731005), M-22 (MAFF 731006), M-23 (MAFF 731007); Tottori Prefecture, Tohaku, Hokuei, Tottori Prefecture Horticultural Research Center, on *P*. *pyrifolia* var. *culta* 'Nijisseiki', Jul 1999, by F. Yasuda, 9901A (MUCC 2151), 9903A (MUCC 2152), 9904C (MUCC 2153); Hokkaido, Esashi, on *Fragaria* × *ananassa* 'HS-138', Aug 2007, by T. Misawa, E-11 (MAFF 242310).

Notes: Simmons (1993, 2007) and Woudenberg *et al.* (2015) have recently re-introduced the name *A*. *gaisen* Nagano, with the brief drawings in Nagano (1920) designated as the lectotype (Fig. 3.4). However, Nagano (1920) explicitly proposed this epithet as a "provisional name" in Japanese without sufficient descriptions such as conidial morphology. For this reason, contemporary Japanese researchers treated the name *A*. *gaisen* Nagano as "nom. invalid." (Hara 1925; Miura 1928; Tanaka 1933). Bokura (1924) accepted this name with reluctance and re-described the species *A*. *gaisen* Nagano with a detailed morphological description. In addition, Hara (1925) had reported *A*. *gaisen* Nagano with its morphology, but all of his descriptions were simply cited from those in Bokura (1924). Based on the criteria described in International Code of Nomenclature for algae, fungi, and plants (ICN) article 46.5 (McNeill *et al.* 2012; http://www.iapt-taxon.org/nomen/main.php), *A*. *gaisen* Nagano ex Bokura is therefore appropriately considered the correct name for this fungus. *Alternaria kikuchiana* S. Tanaka, which was inaccurately cited as *A*. *kikuchii* S. Tanaka in Hara (1936), inevitably corresponds to a later synonym of *A*. *gaisen*. As to the two additional taxa having pathogenicity to Chinese pear, Tanaka (1933) considered *A*. *nashi* Miura (as *Macrosporium nashi* Miura) and *A*. *bokurai* Miura [synonymized to *A*. *manshurica* Hara (Hara 1936)], to be different species because their host cultivars are not susceptible to *A*. *gaisen*. Within the species *A*. *gaisen*, two formae speciales, *A*. *gaisen* f. sp. *pyri* producing the AK-toxin and f. sp. *fragariae* producing the AF-toxin, are newly proposed in this paper.

Fig. 3.4. Original illustration in Nagano (1920) designated as the lectotype of *Alternaria gaisen* by Simmons (2007).

DISCUSSION

Formal designations for the *Alternaria* pathogen of black leaf spot on strawberry, namely, the strawberry pathotype of *A*. *alternata* (Nishimura 1980) or *A*. *alternata* f. sp. *fragariae* (Dingley 1970), were proposed because the morphology of this pathogen meets the criteria given for *A*. *alternata* (Simmons 1967). However, the species definition for *A*. *alternata* is now considered to be incomplete (Simmons 1999, 2007). The pathogen was first reported from Japan by Watanabe & Umekawa (1977), whose isolates MAFF 731001–731007 have been recently located and deposited. To reveal taxonomic relationships within sect. *Alternaria* based on an integrated species criterion (Nishikawa & Nakashima 2013), we performed morphological and molecular characterizations of the fungus using the above seven reliable isolates.

We found that the isolates originating from strawberry and Japanese pear, which have toxicity to leaves of Japanese pear 'Nijisseiki' (Fig. 3.2A, B), formed conidia after 7 d incubation on PCA. Compared with the conidia of *A*. *alternata* isolates, these conidia were larger and arranged in relatively shorter chains without lateral branches (Table 1). According to our detailed morphological observations, we consequently have concluded that the pathogen is morphologically identical to *A*. *gaisen* as defined by Simmons (1999, 2007) and distinguishable from *A*. *alternata* on the basis of sporulation pattern and conidial size. All previous morphological studies of the fungus concluded that the strawberry black leaf spot pathogen was indistinguishable from *A*. *alternata*, although Watanabe & Umekawa (1977) also noted that the *A*. *alternata*-like species on strawberry has shorter conidial chains than those of *A*. *alternata*. Although our examined isolates contained a few unstable strains, the morphology of caespituli formed freshly on PCA were observable; this allowed us to use the sporulation pattern, which is an important and effective criterion for species delimitation in *Alternaria* (Simmons & Roberts 1993; Simmons 1999), to distinguish the pathogen from *A*. *alternata*.

Phylogenetic analysis using an ITS, *gapdh*, *rpb2*, *tef1*, *Alt a 1*, and *endoPG* concatenated dataset also clearly supported *A*. *gaisen* as a distinct morphospecies (Fig. 3.3), consistent with previous studies (Roberts *et al.* 2000; Andersen *et al*. 2001; Peever *et al.* 2004; Armitage *et al.* 2015;

Woudenberg *et al.* 2015). However, neither the morphological nor the phylogenetic analysis was able to distinguish the two pathotypes identified as *A*. *gaisen* on strawberry and Japanese pear. We thus propose two new species forms according to the trinomial concept of Rotem (1994) and Woudenberg *et al.* (2015), which defines the affinity to a specific host according to the produced toxin: *A*. *gaisen* f. sp. *fragariae* producing AF-toxin and *A*. *gaisen* f. sp. *pyri* producing AK-toxin. Because at least 16 different f. sp. epithets (e.g., *A*. *alternata* f. sp. *cucurbitae*) are included in Simmons (2007) and 1,208 *A*. *alternata* host plant records are known worldwide (Farr & Rossman 2017), further studies on the pathogenicity and host selectivity of *A*. *alternata* strains are needed. Although this third epithet is not governed by the ICN (Art. 4.4 Note 4), we hope that f. sp. epithets will be assigned to enable practical recognition of these different pathogenic strains.

Among the seven known *A*. *alternata* HST producers, *A*. *alternata* f. sp. *citri* pathotype *tangerine* (= *A*. *toxicogenica*) morphologically most closely resembles *A*. *gaisen* in terms of sporulation in short chains (Simmons 1999) and production of HSTs (ACT-toxin) toxic to the leaves of 'Nijisseiki' (Maekawa *et al.* 1984; Kohmoto *et al.* 1993). Moreover, all three HSTs (ACT-, AF-, and AK-toxins) have a common structural moiety, 9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid (Nakashima 1982; Nakashima *et al*. 1985; Nakatsuka *et al.* 1986; Kohmoto *et al.* 1993), and the three taxa share genes encoding enzymes for its biosynthesis (Tanaka *et al.* 1999; Masunaka *et al.* 2000; Tanaka & Tsuge 2000; Hatta *et al.* 2002). These facts would suggest that *A*. *alternata* f. sp. *citri* pathotype tangerine should also be consolidated into a congeneric species, but, interestingly, the results of our phylogenetic analysis do not support this hypothesis (Fig. 3.3).

As detailed above, we investigated the taxonomy of the *A*. *alternata*-like pathogen on strawberry based on morphology and molecular analyses, which revealed that *A*. *gaisen* is the correct taxon. Our results should aid future investigations of the evolution of pathogenicity and host specialization of *A*. *alternata* and related taxa.

DISCLOSURE

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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Chapter 4

Taxonomic characterization and experimental host ranges of four newly recorded species of *Alternaria* from Japan

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ABSTRACT

Alternaria fungi are important plant pathogens. Here, we identified three species new to the Japanese mycoflora: *Alternaria celosiae*, *Alternaria crassa*, and *Alternaria petroselini.* We proposed a new name for *A. celosiae* (E.G. Simmons & Holcomb) Lawrence, Park & Pryor, a later homonym of *A. celosiae* (Tassi) O. Săvul. To characterize these and a fourth morphological taxon, *Alternaria alstroemeriae*, which was recently added to Japan's mycoflora, an integrated species concept was tested. We determined the host range of each isolate using inoculation tests and analyzed its phylogenetic position using sequences of the internal transcribed spacer rDNA. The pathogenicity of our *A. alstroemeriae* isolate was strictly limited to *Alstroemeria* sp. (*Alstroemeriaceae*), but the species was phylogenetically indistinguishable from other small-spored *Alternaria*. *Alternaria celosiae* on *Celosia argentea* var. *plumosa* (*Amaranthaceae*) was also pathogenic to *Amaranthus tricolor*, to *Alternanthera paronychioides* and weakly to *Gomphrena globosa* (all *Amaranthaceae*) and formed a clade with the former *Nimbya celosiae. Alternaria crassa* on *Datura stramonium* (*Solanaceae*) was also pathogenic to *Brugmansia* × *candida* and *Capsicum annuum* in *Solanaceae*, but not to other confamilial plants; phylogenetically it belonged to a clade of large-spored species with filamentous beaks. Morphological similarity, phylogenetic relationship, and experimental host range suggested that *A. crassa*, *Alternaria capsici*, and *Alternaria daturicola* were conspecific. *Alternaria petroselini* on *Petroselinum crispum* (*Apiaceae*) was pathogenic to five species in the tribe *Apieae* as well as representatives of *Bupleureae*, *Coriandreae*, *Seliaeae*, and *Scandiceae* in *Apiaceae*. Both phylogeny and morphology suggested conspecificity between *A. petroselini* and *Alternaria selini*.

Keywords: *Alternaria alstroemeriae*, *Alternaria celosiae*, *Alternaria crassa*, *Alternaria petroselini*, host range

INTRODUCTION

The nearly 400 species of *Alternaria* and several allied fungal genera are widely distributed throughout the world; Simmons (2007) described nearly 300 species and recognized an additional 100 previously published taxa that he could not examine. *Alternaria* are commonly found on both living and dead plants (Kirk *et al.* 2008). Most species cause plant diseases (Yu 2001) and are important plant pathogens. An identification key to *Alternaria* and its allied genera based on their morphological characteristics and sporulation patterns on culture media was published by Simmons (2007); these criteria have subsequently been widely used by plant pathologists. However, most known species were recognized only on the basis of complicated morphology, without pathological or phylogenetic examination. The morphological variability and lack of distinguishing features in *Alternaria*-like fungi complicate their identification and diagnosis in infected plants. In contrast, verifying host range through inoculation tests, such as by Neergaard (1945), remains a valuable tool to identify these pathogens. Consequently, these species have been classified based on their observed host plants rather than by their morphology, resulting in new species descriptions from around the world.

In the last decade, phylogenetic analyses using nuclear and mitochondrial DNA sequences have supported broad morphological categories for *Alternaria*, including small- and large-spored groups (Pryor & Gilbertson 2000; Chou & Wu 2002; Pryor & Bigelow 2003; Peever *et al.* 2004; Hong *et al.* 2005a). However, the molecular approach to species identification remains disputed. In particular, for small-spored *Alternaria* species, Andrew *et al.* (2009) showed that multilocus phylogenetic analysis did not resolve morphological species. In contrast, a few species were molecularly identified as distinct using only sequences of the ribosomal ITS (including the 5.8S rRNA gene); for example, *Alternaria hungarica* B. Tóth, J. Varga, M. Csősz, E.G. Simmons & R.A. Samson was separate from *Alternaria mouchaccae* E.G. Simmons, *Alternaria molesta* E.G. Simmons, *Alternaria geniostomatis* E.G. Simmons & C.F. Hill and *Alternaria soliaridae* E.G. Simmons (Tóth *et al.* 2011). Thus, neither morphology, phylogeny, nor pathology alone is sufficient to delimit species of *Alternaria*.

Here, we propose a new species concept that incorporates morphological taxonomy, phylogeny, and host range to delimit species of *Alternaria* and allies. This integrated species concept provides a more comprehensive assessment of species limits in this group than Simmons' morphological concept alone. Furthermore, this concept can assist in identifying and diagnosing plant diseases in Japan, where these species remain insufficiently studied; only 56 *Alternaria* species have been recorded there (Katumoto 2010). In this paper, the morphology and experimental host ranges of four species of *Alternaria* newly recorded from Japan are described, and sequences of their ITS regions provide reference data. In addition, some taxonomic suggestions based on the integrated concept are made.

MATERIALS AND METHODS

Fungal collection and isolation

Leaves of *Alstroemeria* sp. (alstroemeria; *Alstroemeriaceae*), *Celosia argentea* var. *plumosa* (feather cockscomb; *Amaranthaceae*), *Datura stramonium* (jimsonweed; *Solanaceae*), and *Petroselinum crispum* (parsley; *Apiaceae*) showing disease symptoms (see descriptions below) were collected in Nagano prefecture, Kanagawa prefecture, the Tokyo metropolitan area, and Shizuoka prefecture, Japan, respectively. Alternarioid conidia on spots of diseased leaves were suspended in sterilized water and spread on 2 % aqueous agar medium using a flame-sterilized microspatula. After incubation at 20 °C for 24 h, individual germinating conidia were confirmed under a light microscope then transferred to potato–carrot agar (PCA; Simmons 2007) using a flame-sterilized microtube (Nakashima *et al.* 2011). All purified cultures originating from single conidia were deposited in the Genebank, National Institute of Agrobiological Sciences (MAFF), Tsukuba, Japan.

Morphological observation

For microscopic observations, the isolates were newly plated onto V8 juice agar (V8; Simmons 2007) and/or PCA. Plates were maintained at 25 °C in the dark for 7–14 d. The growing colonies were scratched and spread using a flame-sterilized microspatula, and aerial mycelia were removed to observe sporulation more easily. After 12–24 h of pre-incubation in unsealed Petri dishes at 25 °C under blacklight blue bulbs, the plates were incubated at 20 °C in the dark, and sporulation pattern and conidial morphology were observed once per day for 7 d. This sporulation method was introduced by Tohyama (1993) and partially modified (Nishikawa 2008) to obtain diagnostic conidia and conidial catenation comparable to those of Simmons' standard conditions (2007). Caespituli formed on the medium were mounted with Shear's mounting fluid [300 mL aqueous potassium acetate (2 %), 120 mL glycerin, and 180 mL ethanol (95 %)] and observed under a light microscope. The morphology of 100 conidia and 30 conidiophores were examined at 400× magnification.

Culture characteristics on potato–dextrose agar

Each isolate was plated onto potato–dextrose agar (PDA; 40 g potato, 20 g dextrose, 20 g agar in 1.0 L distilled water) plates. After incubation in the dark for 7 d at 25 °C, five colonies of each isolate were measured and the culture characteristics were recorded.

Inoculation tests

Conidia that artificially sporulated on V8 media were washed with sterile distilled water containing 0.02 % polyoxyethylene (20) sorbitan monolaurate (Wako Pure Chemicals, Osaka, Japan) and rubbed with a sterilized plastic spreader. Released conidia were harvested and used as inocula. Concentrations of each conidial suspension were adjusted to approximately 1.9×10^6, 4.2×10^4, 1.6×10^4, and 5.7×10^5 conidia/mL for the *Alternaria* samples isolated from *Alstroemeria* sp., *C. argentea* var. *plumosa*, *D. stramonium*, and *P. crispum*, respectively. Each inoculum was sprayed onto matured leaves of potted plants (at least 3–5 replicates) of 6–13

species related to its original host (Table 4.1). All inoculated plants were kept in an incubator under moist conditions at 20 °C.

Virulent phenotypes were evaluated 9 or 10 days post-inoculation (dpi) using the scale described by Chaerani *et al.* (2007) (0: no visible lesions on leaf; 1: up to 10 % of leaf area affected; 2: 11–25 %; 3: 26–50 %; 4: 51–75 %; 5: more than 75 % of leaf area affected or leaf abscised) and the means were calculated as disease severity. Student's *t*-tests were performed to analyze statistically each disease severity mean. Furthermore, we observed changes in lesion diam and presence or absence of sporulation on the lesion to evaluate pathogenicity. Symptoms were continuously observed until 30 dpi.

DNA and phylogenetic analyses

Total DNA was extracted from mycelia in agar discs using UltraClean™ Microbial DNA Isolation Kits (Mo-Bio Laboratories, Carlsbad, CA, USA). The ITS-1 and ITS-2 regions, including the intervening 5.8S rDNA, were amplified using *Taq* DNA polymerase (Bioline, London, UK) and primers V9G (de Hoog & Gerrits van den Ende 1998) and ITS4 (White *et al.* 1990) in a TP650 thermal cycler (TaKaRa, Ohtsu, Japan) as described by Cheewangkoon *et al.* (2008). Both strands were sequenced using the BigDye Terminator v.3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Carlsbad, CA, USA) in the TP650 thermal cycler. The products were purified with Sephadex G-50 medium (GE Healthcare, Buckinghamshire, UK) and Multiscreen plates (Millipore, Billerica, MA, USA) (Crous *et al.* 2010). Sequences were analyzed using a3730xl Genetic Analyzer (Applied Biosystems) at the Life Science Research Center, Mie University, Mie, Japan. Sequences were deposited in the DNA data bank of Japan (DDBJ; accession numbers AB678214–AB678217). Partial sequences of three protein-coding genes were also obtained and deposited in DDBJ as reference data for future studies: *act* (Carbone and Kohn 1999), *Alt a 1* (Hong et al. 2005b), and *gapdh* (Berbee *et al.* 1999).

Complementary strands were assembled using MEGA5 (Tamura *et al.* 2011). An NCBI BLAST search was performed to find relatives of the newly sequenced taxa. To analyze the relationships of our isolates to known *Alternaria*-like species, we aligned our sequence data to

Table 4.1. Host ranges of Japanese isolates of *Alternaria* species in inoculation tests.

Inoculum	Plant group	Plant species	Disease severity [a]	*t* statistic [b]	*Df* [c]	Lesion diameter [d]	Notes
Alternaria alstroemeriae (MAFF241374)	*Liliales:* *Alstroemeriaceae*	*Alstroemeria* sp.	3.7			+	Pathogenic; distinct spots, rich sporulation within 10 dpi.
	Liliaceae	*Lilium longiflorum*	0	5.066****	7	−	Non-pathogenic; no symptoms or sporulation by 30 dpi.
		Tulipa gesneriana	0	7.416*****	10	−	Non-pathogenic; no symptoms or sporulation by 30 dpi.
	Asparagales: *Amaryllidaceae*	*Allium fistulosum*	0	9.811*****	14	−	Non-pathogenic; no symptoms or sporulation by 30 dpi.
	Asparagaceae	*Asparagus officinalis*	0	8.685*****	12	−	Non-pathogenic; no symptoms or sporulation by 30 dpi.
	Hyacinthaceae	*Hyacinthus orientalis*	0	5.066****	7	−	Non-pathogenic; no symptoms or sporulation by 30 dpi.
Alternaria celosiicola (MAFF243058)	*Amaranthaceae:* *Amaranthoideae*	*Celosia argentea* var. *cristata*	4.6			+	Pathogenic; distinct spots, rich sporulation, defoliation by 10 dpi.
		C. argentea var. *plumosa*	4.7	−0.478	26	+	Pathogenic; distinct spots, rich sporulation, defoliation by 10 dpi.
		Amaranthus tricolor	4.4	0.408	24	+	Pathogenic; distinct spots, rich sporulation, defoliation by 10 dpi.
	Gomphrenoideae	*Gomphrena globosa*	3	3.607****	24	+	Weakly pathogenic; distinct spots by 3 dpi, poor sporulation by 30 dpi.
		Alternanthera paronychioides	4.5	0.203	22	+	Pathogenic; indistinct spots, rich sporulation, defoliation by 10 dpi.
	Betoideae	*Beta vulgaris*	0.2	13.720*****	22	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.
	Chenopodioideae	*Spinacia oleracea*	0.1	14.426*****	22	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.

Table 4.1. (Continued).

Inoculum	Plant group	Plant species	Disease severity [a]	*t* statistic [b]	*Df* [c]	Lesion diameter [d]	Notes
Alternaria crassa (MAFF243056)	*Solanaceae: Solanoideae Datureae*	*Brugmansia × candida*	2.3			+	Pathogenic; distinct pale brown spots, sporulation by 9 dpi.
	Capsiceae	*Capsicum annuum*	4.2	−2.685**	11	+	Strongly pathogenic; indistinct spots, rich sporulation, defoliation by 7 dpi.
	Physaleae	*Physalis alkekengi*	0.3	5.292****	7	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.
	Solaneae	*Solanum melongena*	0.4	4.327****	11	−	Non-pathogenic; small spots rare by 30 dpi, no sporulation.
		Solanum lycopersicum	1.2	3.564**	7	±	Non to weakly pathogenic; spots by 7 dpi but no sporulation by 30 dpi.
	Nicotianoideae	*Nicotiana tabacum*	0	12.663*****	9	−	Non-pathogenic; no symptoms or sporulation by 30 dpi.
	Petunioideae	*Petunia × hybrida*	0.2	7.139*****	11	±	Non to weakly pathogenic; spots rare by 10 dpi and weak by 30 dpi, no sporulation.
Alternaria petroselini (MAFF243057)	*Apiaceae: Apioideae Apieae,*	*Petroselinum crispum*	3.8			+	Pathogenic; distinct spots to leaf blight, rich sporulation by 10 dpi.
		Ammi majus	2.9	1.448	28	+	Pathogenic; spots, rich sporulation, defoliation by 5 dpi.
		Anethum graveolens	4.4	−1.099	28	+	Pathogenic; distinct spots to leaf blight, rich sporulation by 5 dpi.
		Apium graveolens	3.2	0.923	28	+	Pathogenic; distinct spots to leaf blight, rich sporulation by 10 dpi.
		Foeniculum vulgare	3.3	0.809	28	+	Pathogenic; distinct spots to leaf blight, rich sporulation by 5 dpi.

Table 4.1. (Continued).

Inoculum	Plant group	Plant species	Disease severity [a]	*t* statistic [b]	*Df* [c]	Lesion diameter [d]	Notes
	Bupleureae	*Bupleurum rotundifolium*	2.6	1.938*	28	+	Pathogenic; distinct spots to leaf blight, sporulation by 7 dpi.
	Careae	*Carum carvi*	0	7.722*****	28	−	Non-pathogenic; tip-burn rare, poor sporulation by 30 dpi.
	Coriandreae	*Coriandrum sativum*	3.5	0.54	28	+	Pathogenic; distinct spots to leaf blight, rich sporulation by 10 dpi.
	Oenantheae	*Cryptotaenia japonica*	0	7.722*****	28	−	Non-pathogenic; no symptoms or sporulation by 30 dpi.
	Scandiceae *Daucinae*	*Cuminum cyminum*	5	−1.879*	24	+	Strongly pathogenic; leaf blight, rich sporulation by 5 dpi.
		Daucus carota	0.6	8.489*****	38	±	Non-pathogenic; indistinct leaf blight rare, no to poor sporulation by 15 dpi.
	Scandicinae	*Anthriscus cerefolium*	3.7	0.18	28	+	Pathogenic; distinct spots to leaf blight, rich sporulation by 5 dpi.
	Seliaea	*Angelica keiskei*	2.2	2.598**	28	+	Weakly pathogenic; distinct spots to leaf blight by 10 dpi, no or poor sporulation by 30 dpi.

[a] Mean disease severity at 9 d post-inoculation (dpi) rated on a 0–5 scale (0 = no visible lesions, 1 = <10 % leaf area affected, 2 = 11–25 %, 3 = 26–50 %, 4 = 51–75 %, 5 = >75 % or defoliated).

[b] Student's two-tailed *t* statistic between the disease severity means of the host plant and the related test host. *, P <0.1; **, P <0.05; ***, P <0.01; ****, P <0.005; *****, P <0.001).

[c] Degrees of freedom (*Df*) [$(n_1-1) + (n_2-1)$] for Student's *t*-test.

[d] Change within 30 dpi; +, increased; −, unchanged.

the 72 sequences of Lawrence *et al.* (2011; TreeBASE study S11665) using MUSCLE (Edgar 2004) in MEGA5 with default settings and adjusted the alignment manually. Aligned positions with gaps or missing data were eliminated using the COMPLETE DELETION option. Phylogenetic trees were constructed using maximum parsimony in MEGA5 based on the close-neighbour-interchange algorithm with SEARCH LEVEL 3 and random sequence addition (10 replicates) to obtain the initial. Support for branches was gauged with 500 bootstrap (BS) replicates; values >60 % are shown on the resulting tree (Felsenstein 1985). *Exserohilum pedicellatum* was the outgroup.

RESULTS

Taxonomy

Based on their morphology on host plants and on V8 or PCA media, the following four species of *Alternaria*, including three newly added to the Japanese mycoflora, are described.

Alternaria alstroemeriae E.G. Simmons & C.F. Hill, in Simmons, Alternaria *an Identification Manual*: 444, 2007. Fig. 4.1.

Specimen and isolate examined: on *Alstroemeria* sp., **Japan**, Matsumoto, Nagano Prefecture, January 2008, by *N. Yamagishi* (deposited culture: MAFF 241374; deposited sequences, ITS: AB678214, *act*: AB744038, *Alt a 1*: AB744031, *gapdh*: AB744034).

Culture characteristics on PDA: Colonies fast-growing, reaching 49 and 75 mm in diam at 25 °C after 5 and 7 d, respectively.

Notes: Yamagishi *et al.* (2009) reported the symptoms and morphology of this fungus. This specimen is a new record for Japan; the species was previously known only from the type locality in Australia (Simmons 2007). Based on the BLAST results, this isolate had sequences similar to other members of 'alternata sp.-group' but had phylogenetically unique *Alt a 1*

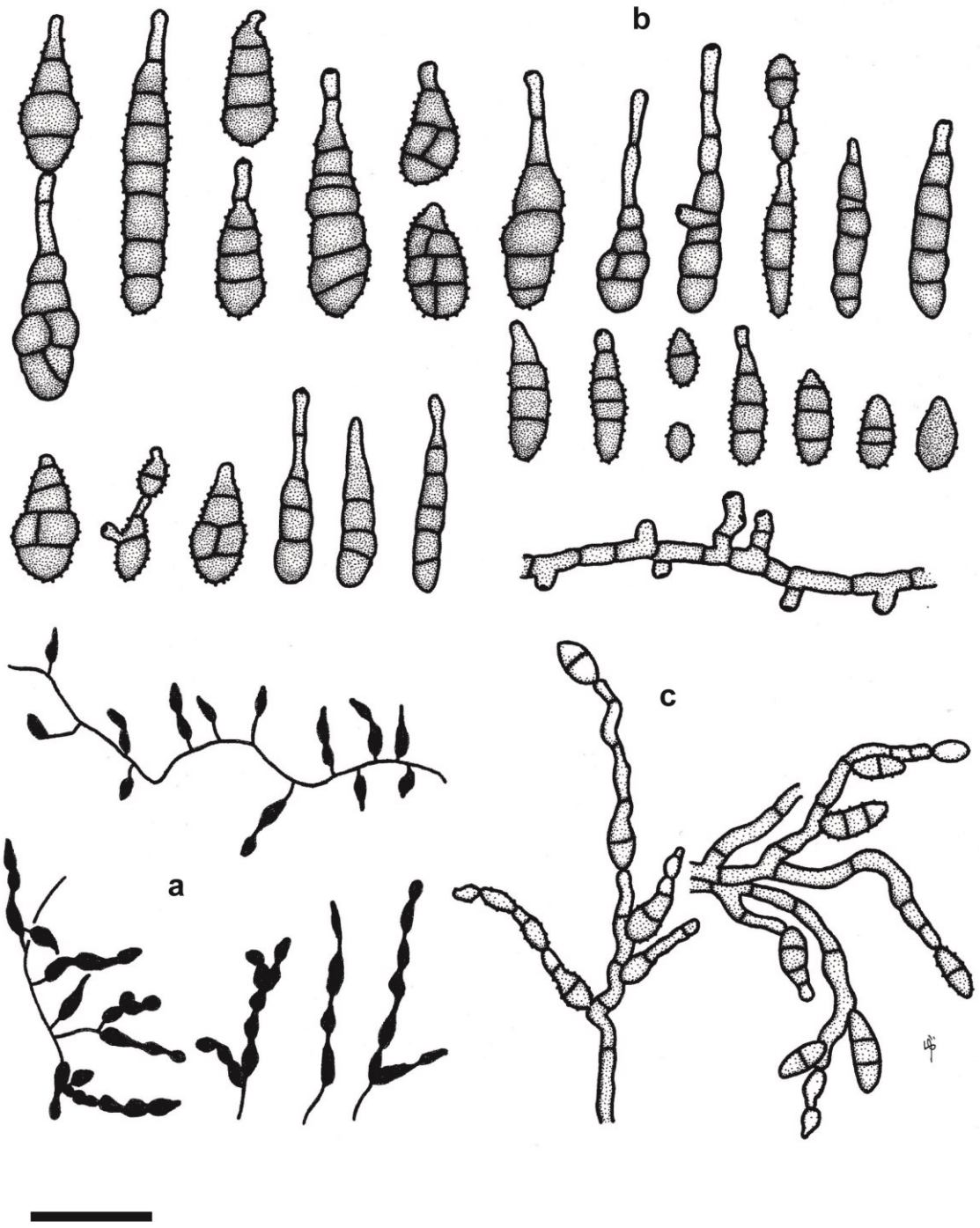

Fig. 4.1. *Alternaria alstroemeriae* (MAFF241374) on potato-carrot agar medium. **a.** Sporulation pattern (opaque). **b.** Conidia and conidiophores. **c.** Sporulation submerged in media. Bar = 25 µm.

sequences.

Alternaria celosiicola Jun. Nishikawa & C. Nakash., ***nom. nov.*** MycoBank no.: MB800983. Figs 4.2, 4.3a.

Basionym: *Nimbya celosiae* E.G. Simmons & Holcomb, in Simmons, *Mycotaxon* **55**: 144, 1995. ≡ *Alternaria celosiae* (E.G. Simmons & Holcomb) Lawrence, Park & Pryor, *Mycol. Prog.* **11**: 811, 2012. nom. illeg. (ICN Art. 53.1), later homonym of *A. celosiae* (Tassi) O. Săvul., Herb. *Mycol. Rom.*: fasc. 30, no. 1489, 1950.

Leaf spots circular to subcircular, distinct at borders, 2–10 mm in diam, confluent, brown to dark brown with reddish margin, often with a yellowish halo. Conidia on V8 usually solitary, pale brown to brown, subcylindrical to narrowly ellipsoid, (78.8–)117.5–755 µm in total length, surface commonly smooth; conidial bodies (42–)78.8–180 × 9.5–26.3 µm with 2–17 transverse and 0–5 longitudinal septa, including distosepta; lumina usually distinct, octagonal to round; filamentous beaks straight to slightly curved, (25–)48.8–575 × 2–3.8 µm, subhyaline to pale brown, multiseptate, unbranched, often swollen at the apex. Conidiophores short and broad, 25–68.8 × 5–7 µm. Conidia on lesion (187.5–)227.5–430 µm in total length; conidial bodies (67.5–)87.5–172.5 × (12.5–)16.3–26.3 µm with 8–15 transverse and 0–1 longitudinal septa, including distosepta; beaks 120–285 × 2–3 µm, unbranched.

Specimen and isolate examined: on *Celosia argentea* L. var. *plumosa* Voss, **Japan**, Fujisawa, Kanagawa Prefecture, June 26 2006, by *S. Masugi & Y. Makizumi* (deposited culture: MAFF 243058; deposited sequences, ITS: AB678217, *act*: AB744036, *Alt a 1*: AB744029, *gapdh*: AB744033).

Culture characteristics on PDA: Colonies fast-growing, reaching 50 and 71 mm in diam at 25 °C after 5 and 7 d, respectively; cottony with white to dark green (primarily pale) aerial mycelia, reverse center black to dark green; no pigment released into media (Fig. 4.3b); sporulation sparse.

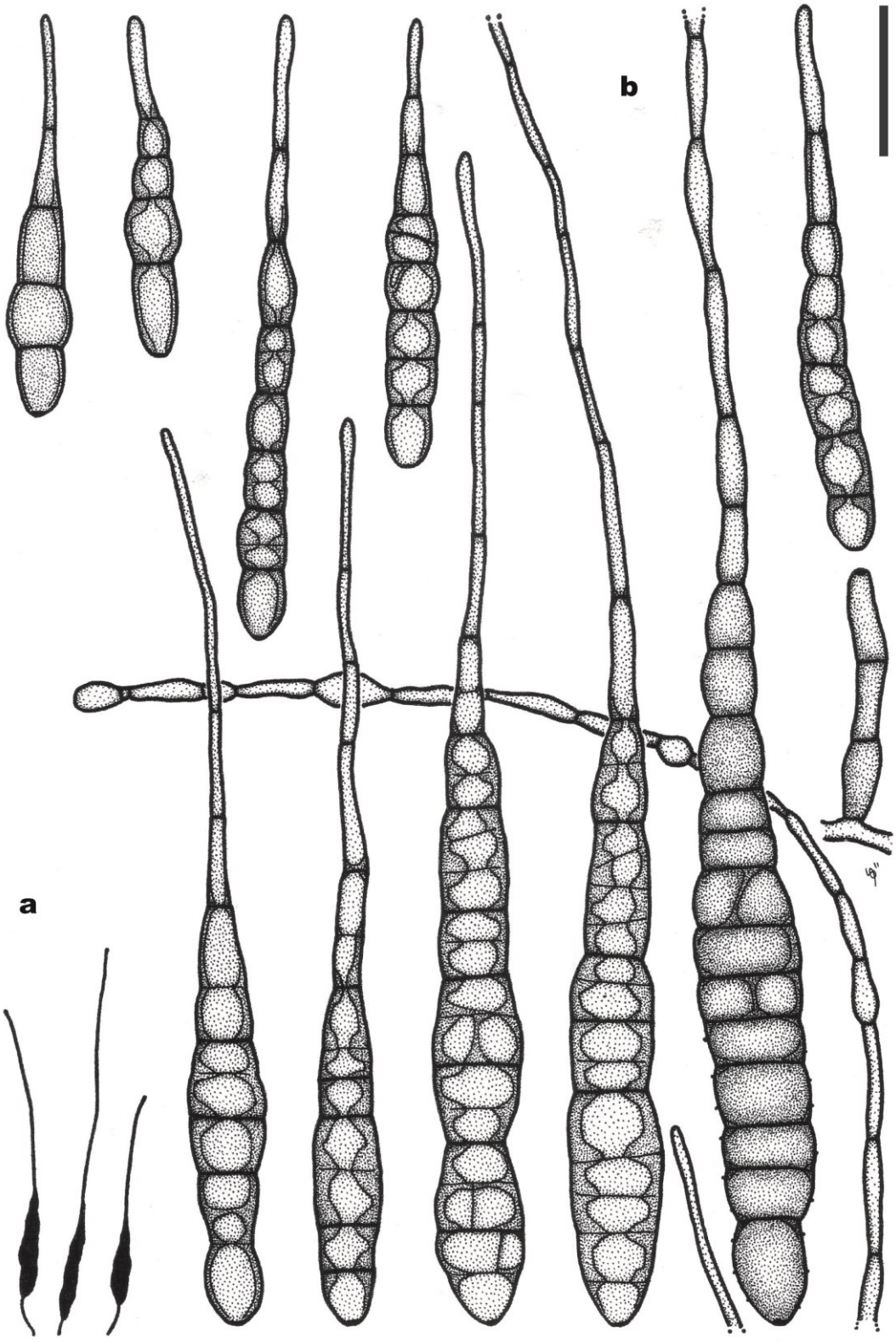

Fig. 4.2. *Alternaria celosiicola* (MAFF243058) on V8 medium. **a.** Sporulation pattern (opaque). **b.** Conidia and conidiophore. Bar = 25 µm.

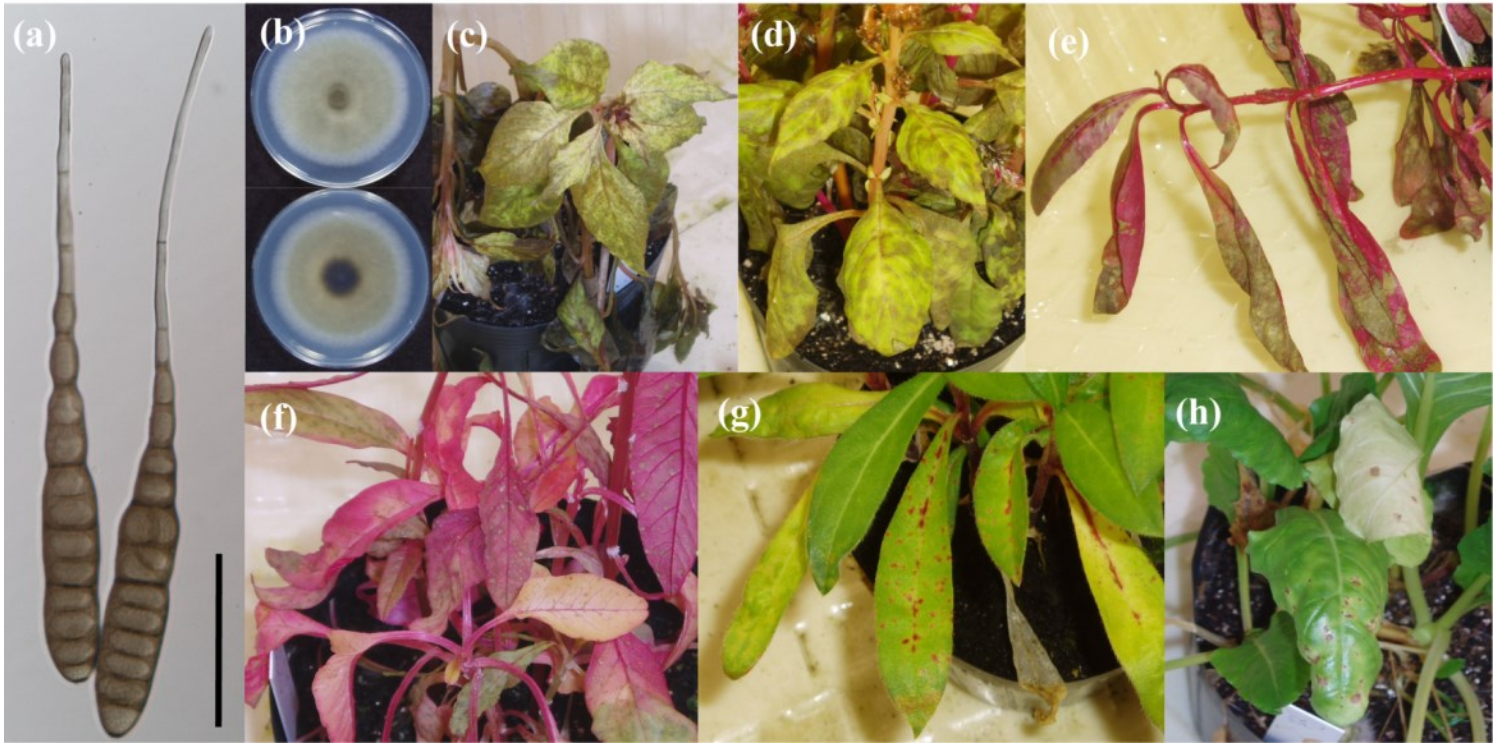

Fig. 4.3. Morphology and symptomatology of an isolate of *Alternaria celosiicola* from Japan (MAFF243058). **a.** Conidia on V8 medium. Bar = 50 µm. **b.** Culture on potato–dextrose agar (upper, surface view; lower, bottom view). **c.** Leaves of *Celosia argentea* var. *cristata* at 5 d post-inoculation (dpi). **d.** Leaves of *Celosia argentea* var. *plumosa* at 4 dpi. **e.** Leaves of *Alternanthera paronychioides* at 6 dpi. **f.** Leaves of *Amaranthus tricolor* at 4 dpi. **g.** Leaves of *Gomphrena globosa* at 30 dpi. **h.** Leaves of *Beta vulgaris* at 30 dpi.

Notes: This species is a new record for Japan. The BLAST results indicated that this isolate had sequences similar to the ex-type strain E.G.S. 42-013 of *A. celosiae* (E.G. Simmons & Holcomb) Lawrence, Park & Pryor (ITS: JN383497, *act*: JQ671716, *Alt a 1*: JN383512, *gapdh*: JN383478).

Alternaria crassa (Sacc.) Rands, *Phytopathology* **7**: 337, 1917. Figs 4.4, 4.5a.

≡ *Cercospora crassa* Sacc., *Michelia* **1**(no. 1): 88, 1877.

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

= *Cercospora daturae* Peck, *Rep. N.Y. St. Mus. Nat. Hist.* **35**: 140, 1884.

= *Macrosporium cookei* Sacc., *Syll. Fung.* **4**: 530, 1886.

= *Macrosporium daturae* Fautrey, *Rev. Myc.* 76, 1894.

= *Alternaria daturae* (Fautrey) Bubák & Ranoj., *Fungi Imperfecti Exsicc. Fasc.* **14**: no. 694, 1909.

= *Alternaria cookei* (Sacc.) Bremer, Işmen, Karel & Özkan & M. Özkan, *Rev. Fac. Sci. Istanbul, ser. B.*, **13**: 42, 1948.

Leaf spots straw-yellow to pale brown with gray center, circular to irregular, vein-limited, distinct at borders, scattered, enlarged and confluent. Conidia on V8 solitary to rarely in chains of immature conidia after 5–7 d. Conidia pale brown, ellipsoid to subcylindrical, (95.5–)260–587 µm in total length, surface smooth; conidial bodies (30–)55–101.3 × (6.3–)13–22.5 µm with (2–)5–11 transverse and (0–)2–8 longitudinal septa; false beaks unbranched to rarely branched, straight and very long, (65.5–)195–515 × 2.5–5.5 µm with (1–)10–27 transverse septa, colored, border inconspicuously from conidial body. Conidiophores short and broad, 25–57.5 × 4.5–7.5 µm. Conidia on lesions shorter than those on V8, (107.5–)150–317.5 × 11.3–22.5 µm in total length; conidial bodies (47–)57.5–87.5 × 11.3–22.5 µm with 5–10 transverse and 0–7 longitudinal septa; false beaks (32.5–)87.5–238.8 × 2–3 µm with multiple indistinct transverse septa and without longitudinal septa. Conidiophores 31.3–62.5 × 3.8–7 µm.

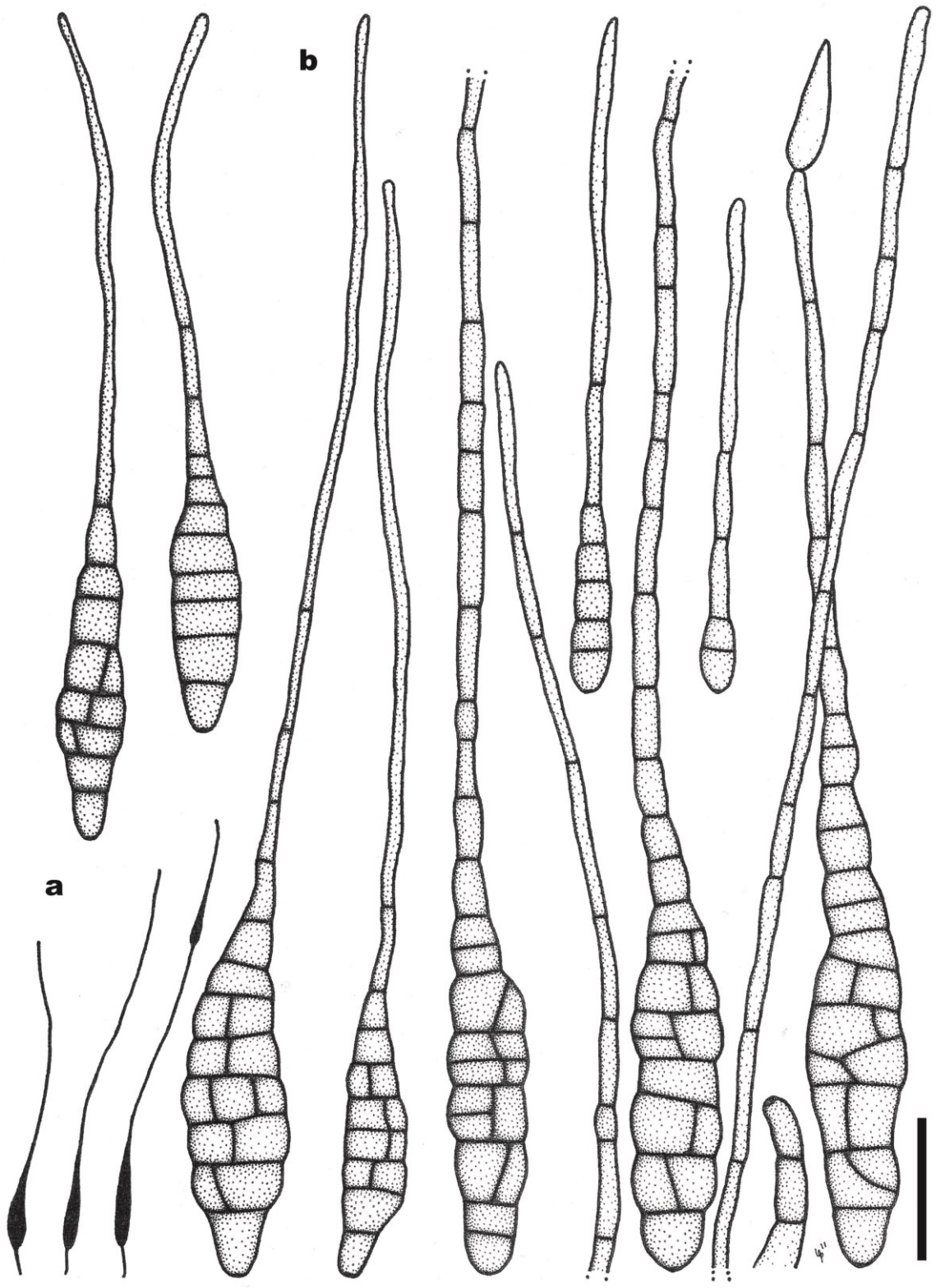

Fig. 4.4. *Alternaria crassa* (MAFF243056) on V8 medium. **a.** Sporulation pattern (opaque). **b.** Conidia and conidiophore. Bar = 25 µm.

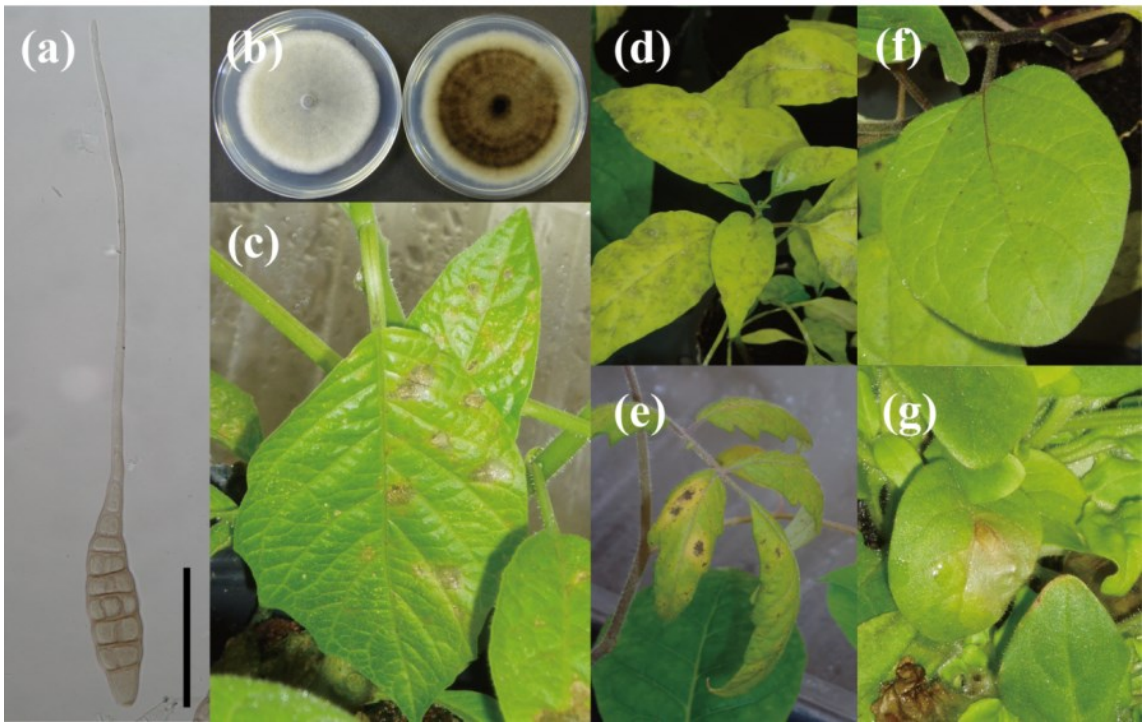

Fig. 4.5. Morphology and symptomatology of an isolate of *Alternaria crassa* (MAFF243056). **a.** Conidia on naturally infected lesion. Bar = 50 µm. **b.** Culture on potato–dextrose agar (left, surface view; right, bottom view). **c.** Leaves of *Brugmansia × candida* at 9 d post-inoculation (dpi). **d.** Leaves of *Capsicum annuum* at 5 dpi. **e.** Leaves of *Solanum lycopersicum* at 8 dpi. **f.** Leaves of *Solanum melongena* at 5 dpi. **g.** Leaves of *Petunia × hybrida* at 29 dpi.

Specimen and isolate examined: on *Datura stramonium* L., **Japan**, Kodaira, Tokyo, July 2000, by *J. Nishikawa* (deposited culture: MAFF 243056; deposited sequences, ITS: AB678215, *act*: AB744035, *Alt a 1*: AB744028, *gapdh*: AB744032).

Culture characteristics on PDA: Colonies fast-growing, reaching 54 and 73 mm in diam at 25 °C after 5 and 7 d, respectively; cottony with white to grayish green aerial mycelia, reverse dark green; no pigment released into media (Fig. 4.5b); sporulation sparse.

Notes: This species is a new record for Japan. Based on the BLAST results, this isolate had sequences similar to those of *A. crassa* isolates DDG Acr1 (ITS: AF229464, *act*: JQ671735, *Alt a*

1: AY563293, *gapdh*: AY278804), *A. crassa* representative strains E.G.S. 44-071 (*Alt a 1*: GQ180088, *gapdh*: GQ180072) and E.G.S. 46-014 (*Alt a 1*: GQ180089, *gapdh*: GQ180073), the ex-type strain E.G.S. 45-075 of *Alternaria capsici* E.G. Simmons (*act*: JQ671747, *Alt a 1*: AY563298, GQ180087, *gapdh*: AY562408, GQ180071), and strain AD46 of *Alternaria daturicola* T.Y. Zhang, G.Z. Zhao & M. Zhang (ITS: AY372685).

Alternaria petroselini (Neerg.) E.G. Simmons, in Ellis, *More dematiaceous Hyphomycetes*: 417, 1976. Figs 4.6, 4.7a.

≡ *Stemphylium petroselini* Neerg., *Zentralbl. Bakteriol. Parasitenk. Infektionskr. 2. Abt.*, **104**: 411, 1942.

≡ *Stemphylium radicinum* var. *petroselini* (Neerg.) Neerg., *Danish species of* Alternaria *&* Stemphylium: 357, 1945.

Leaf spots pale to sooty brown, indistinct at borders, water soaked, visible as leaf blight (Fig. 4.7c). Conidia on PCA mostly solitary, sometimes 2–3 conidia in a chain, lateral branches usually absent after 5–7 d. Conidia dark to yellowish brown, broad ovoid to subsphaeroid, obclavate to long ellipsoid, (15–)26.3–76.3 × (8–)11.3–30 µm with 1–10 transverse and 0–11 (commonly 1–2 per transverse segment) longitudinal septa, surface usually smooth; secondary conidiophores (false beaks) sometimes present, one-celled. Conidiophores frequently geniculate, short to moderately long, relatively narrow, 10–87.5 × 4.5–6.3 µm. Conidia on lesions (19.5–)25.5–60.5 × (10.5–)12.5–25.5 µm with (1–)3–9 transverse and 0–8 longitudinal septa (commonly 1–2 per transverse segment). Conidiophores 17.5–65.5 × 4.5–7.5 µm. Similar characteristics were also seen on V8 medium (data not shown).

Specimen and isolate examined: on *Petroselinum crispum* (Mill.) Fuss, **Japan**, Kakegawa, Shizuoka Prefecture, April 27 2007, by *J. Nishikawa* (deposited culture: MAFF 243057; deposited sequences, ITS: AB678215, *act*: AB744037, *Alt a 1*: AB744030).

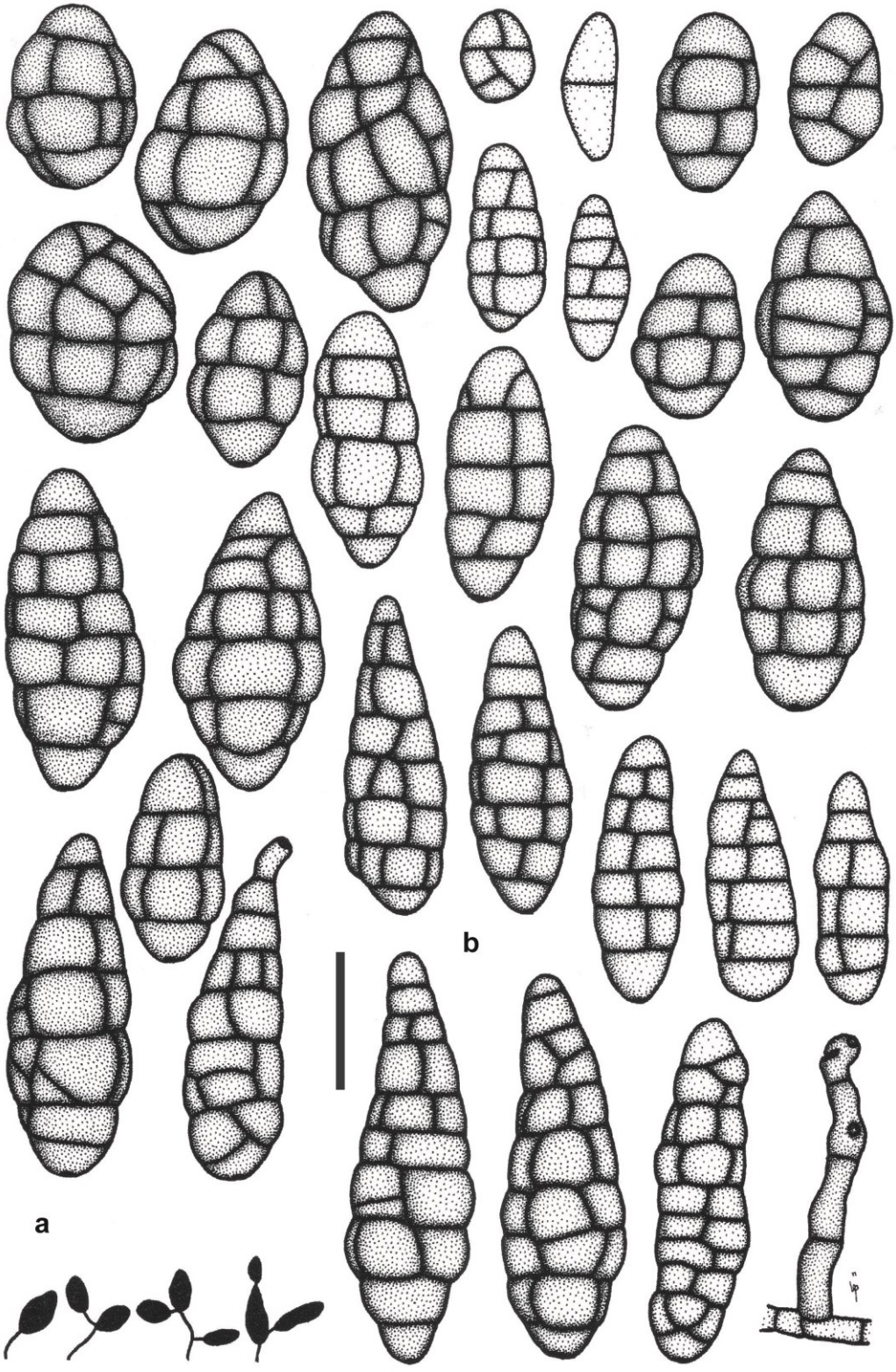

Fig. 4.6. *Alternaria petroselini* (MAFF243057) on potato-carrot agar medium. **a.** Sporulation pattern (opaque). **b.** Conidia and conidiophore. Bar = 25 µm.

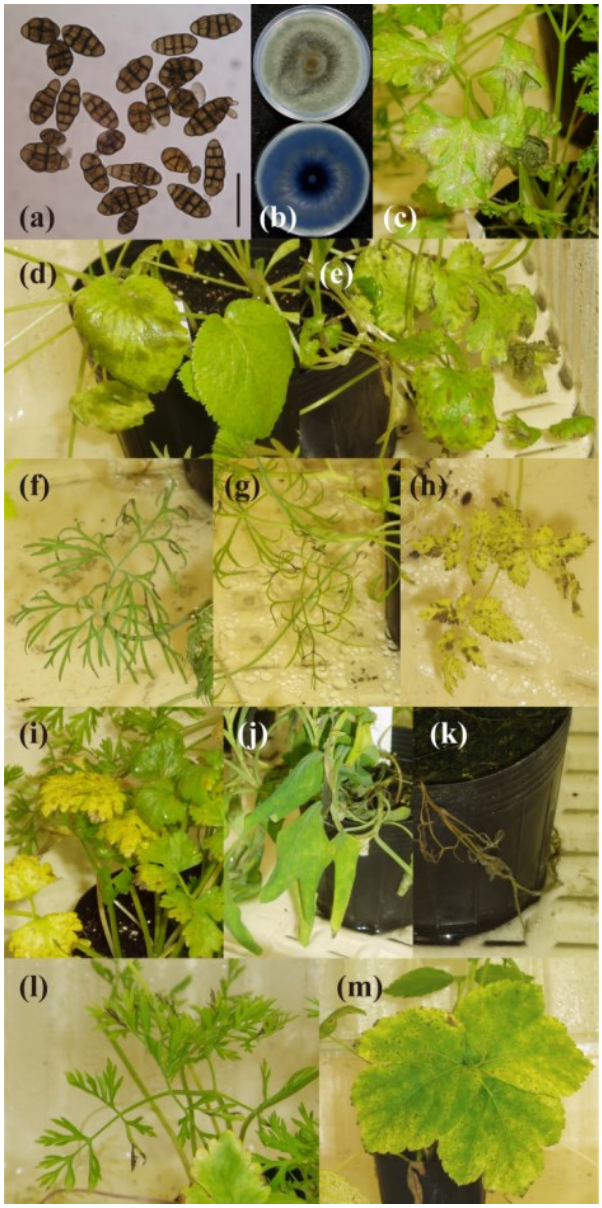

Fig. 4.7. Morphology and symptomatology of an isolate of *Alternaria petroselini* (MAFF243057). **a.** Conidia on V8 medium. Bar = 50 µm. **b.** Culture on potato–dextrose agar (upper, surface view; lower, bottom view). **c.** Leaves of *Petroselinum crispum* at 8 d post-inoculation (dpi). **d.** Leaves of *Ammi majus* at 3 dpi. **e.** Leaves of *Coriandrum sativum* at 9 dpi. **f.** Leaves of *Anethum graveolens* at 3 dpi. **g.** Leaves of *Foeniculum vulgare* at 4 dpi. **h.** Leaves of *Anthriscus cerefolium* at 3 dpi. **i.** Leaves of *Apium graveolens* at 7 dpi. **j.** Leaves of *Bupleurum rotundifolium* at 9 dpi. **k.** Leaves of *Cuminum cyminum* at 7 dpi. **l.** Leaves of *Daucus carota* at 21 dpi. **m.** Leaves of *Angelica keiskei* at 11 dpi.

Culture characteristics on PDA: Colonies fast-growing, reaching 67 and 82 mm in diam at 25 °C after 5 and 7 d, respectively; effuse, with dark green to gray mycelia, reverse center black to dark green; cottony and much clouded; no pigment released into media (Fig. 4.7b); sporulation abundant.

Notes: This species is a new record for Japan. The BLAST results indicated that the sequences of this isolate were closely related to those from the presumably ex-type strain E.G.S. 09-159 of *A. petroselini* (ITS: AF229454, *act*: JQ671677, *Alt a 1*: AY563288) and the ex-type strain E.G.S. 25-198 of *Alternaria selini* E.G. Simmons (ITS: AF229455, *act*: JQ671676, *Alt a 1*: FJ266504, EU139319).

Characterization of the four *Alternaria* species

Phylogenetic analysis

The BLAST search demonstrated that our ITS sequences represented species of *Alternaria sensu lato*. The sequence alignment contained 76 taxa, including one outgroup, and was 463 characters long. Fig. 4.8 shows one of the 468 most-parsimonious trees derived from this analysis. Most of the species-groups of *Alternaria* indicated by Lawrence *et al.* (2012) were recovered. Our culture of *A. alstroemeriae* clustered with the small-spored "alternata sp.-group" (BS = 98 %). The *A. petroselini* isolate formed a sub-clade (BS = 93 %) with an ex-holotype of *A. selini* and *A. petroselini* isolated from *P. crispum* within a "radicina sp.-group" clade (BS = 60 %). *Alternaria celosiicola* grouped with former *Nimbya* species, including the ex-epitype of *A. celosiae* (E.G. Simmons & Holcomb) Lawrence, Park & Pryor (BS = 96 %). Our isolate of *A. crassa* was in the large-spored species with filamentous beaks, "porri sp.-group," although support for the clade was weak (BS = 46 %).

Pathogenicity

The host range and virulence of each examined *Alternaria* species varied (Table 4.1). The Japanese isolate of *A. alstroemeriae* had a restricted host range; it was pathogenic to

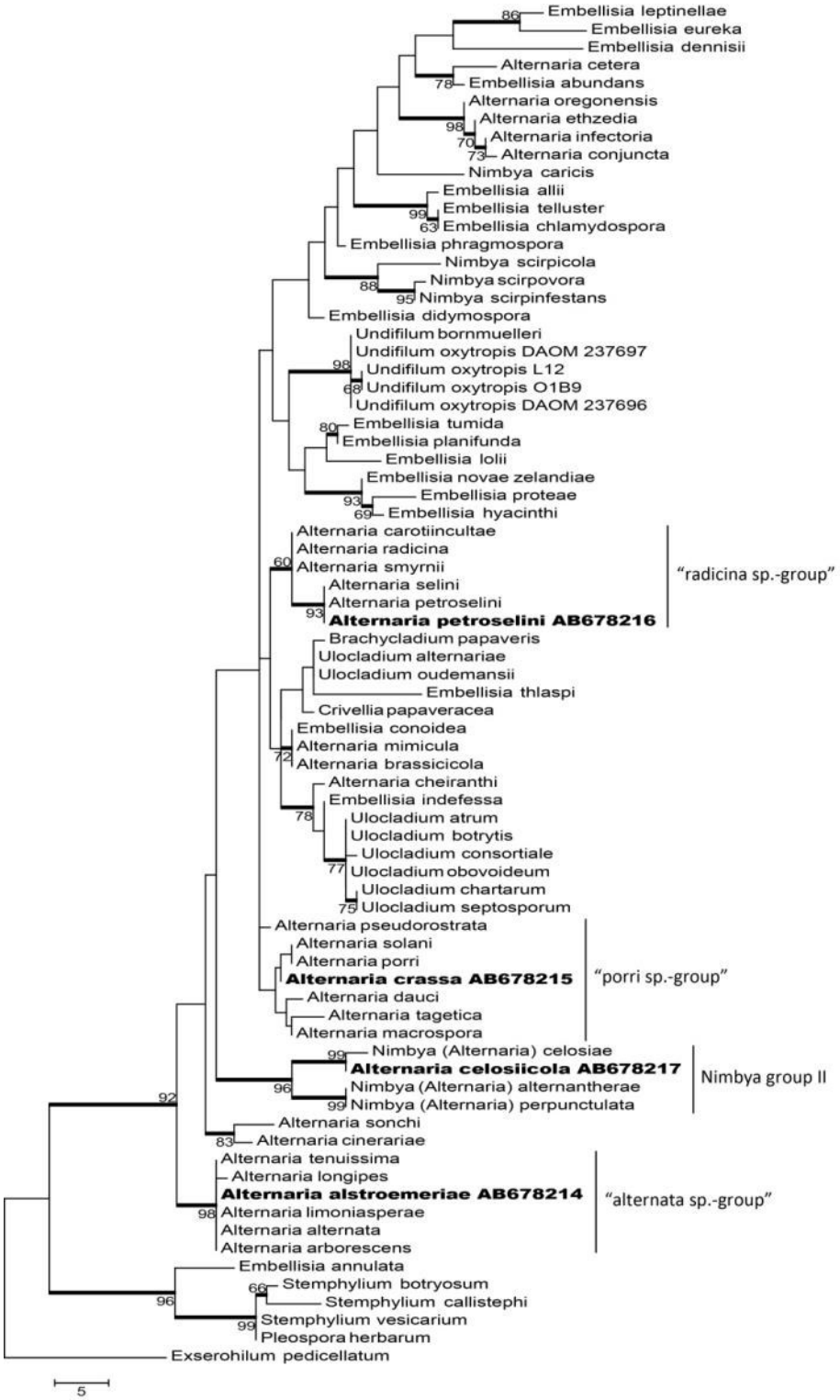

Fig. 4.8. One of 468 most parsimonious trees generated from an analysis of ITS sequences. Bootstrap values >60 % (500 replicates) are shown next to branches. Tree length = 270, consistency index = 0.483, retention index = 0.826, composite index = 0.447. The tree was rooted with *Exserohilum pedicellatum*.

Alstroemeria spp. but not to *Lilium longiflorum* (trumpet lily; *Liliaceae*), *Tulipa gesneriana* (garden tulip; *Liliaceae*), *Allium fistulosum* (welsh onion; *Amaryllidaceae*), *Asparagus officinalis* (garden asparagus; *Asparagaceae*), or *Hyacinthus orientalis* (common hyacinth; *Hyacinthaceae*), all classified or formerly classified in *Liliaceae*. In contrast, the isolate of *A. celosiicola* infected a wide range of amaranthaceous plants (Table 4.1, Fig. 4.3c–h). It had strong pathogenicity to *C. argentea*, *Amaranthus tricolor* (Joseph's coat), and *Alternanthera paronychioides* (smooth joyweed), weak pathogenicity to *Gomphrena globosa* (globe amaranth), and no pathogenicity to *Beta vulgaris* (chard) or *Spinacia oleracea* (spinach). Our isolate of *A. crassa* from *D. stramonium* was inoculated onto several solanaceous species (Table 4.1, Fig. 4.5c–g); it showed strong pathogenicity to *Brugmansia* × *candida* (angel's trumpet; tribe *Datureae*) and *Capsicum annuum* (bell pepper; *Capsiceae*) and moderate pathogenicity to *Petunia* × *hybrida* (petunia; subfamily *Petunioideae*). However, was not pathogenic to *Physalis alkekengi* (Chinese lantern; *Physaleae*), *Solanum lycopersicum* (tomato; *Solaneae*), *Solanum melongena* (eggplant; *Solaneae*), or *Nicotiana tabacum* (common tobacco; subfamily *Nicotianoideae*). Its host range was considered "wide" as it extended beyond the tribe *Datureae*. Our isolate of *A. petroselini* was pathogenic to members of *Apiaceae* tribe *Apieae*, including *P. crispum*, *Ammi majus* (bishop's weed), *Anethum graveolens* (dill), *Apium graveolens* (celery), and *Foeniculum vulgare* (fennel), as well as to *Bupleurum rotundifolium* (thoroughwax; tribe *Bupleureae*), *Coriandrum sativum* (coriander; *Coriandreae*), *Anthriscus cerefolium* (chervil; *Scandiceae*), *Cuminum cyminum* (cumin; *Scandiceae*), and *Angelica keiskei* (ashitaba; *Seliaeae*), but not to *Carum carvi* (caraway; *Careae*), *Cryptotaenia japonica* (mitsuba; *Oenantheae*), or *Daucus carota* (carrot; *Scandiceae*) (Table 4.1, Fig. 4.7c–m). This isolate may have a wide host range within *Apiaceae*.

DISCUSSION

We developed a new integrative species concept that considers morphology, phylogeny, and host range to delimit species of *Alternaria* and its allied genera. To date, most of these species have been defined solely on the basis of complex morphology, which may not distinguish cryptic species and provides little practical insight into pathogenicity. We tested our species concept using four newly recorded species of *Alternaria* in Japan.

Alternaria alstroemeriae

An *Alternaria* species observed on *Alstroemeria* was identified as *A. alstroemeriae* based on the morphological characteristics described by Yamagishi *et al.* (2009). The size of the conidia indicated that the species was a small-spored *Alternaria* (7–63 × 5–18 µm; Yamagishi *et al.* 2009). However, in the phylogenetic analysis of ITS sequences, *A. alstroemeriae* formed a monophyletic clade (BS = 98 %) with strains from the "alternata sp.-group" (Fig. 4.8), despite its unique morphology in having subcylindrical conidia and sporulation submerged in agar substrates (Simmons 2007; Yamagishi *et al.* 2009). Molecular approaches to species identification can sometimes provide misleading results. In particular, Andrew *et al.* (2009) indicated that even multilocus sequence data could not resolve morphological species of small-spored *Alternaria* species. Although its morphology (distinctly subcylindrical conidia) and pathogenicity (host range restricted within *Alstroemeria*) were distinguishable from the other small-spored species (Table 4.1), the validity of *A. alstroemeriae* as a species must be confirmed with additional studies.

Alternaria celosiicola

The six species of *Nimbya* have been identified on several amaranthaceous plants (Simmons 1989; Simmons 1995; Simmons 2004; Gilbert *et al.* 2005; Zhao & Zhang 2005); some were recently reclassified to *Alternaria* based on their morphological characteristics and phylogenetic position (Lawrence *et al.* 2012). However, transferring *Nimbya celosiae* to *Alternaria* resulted in a later homonym, *A. celosiae* (E.G. Simmons & Holcomb) Lawrence, Park & Pryor. Epithet priority belonged to *A. celosiae* (Tassi) O. Săvul., which is regarded as either

incertae sedis or a small-spored *Alternaria* species and clearly distinguishable from *N*. *celosiae* (Zhang 2003; Simmons 2007). Thus, Lawrence's taxonomic treatment of *N*. *celosiae* was not a taxonomic problem but a nomenclatural error, and we propose the new name *A. celosiicola* Jun. Nishikawa & C. Nakash.

Lawrence *et al*. (2012) noted that three former *Nimbya* species—*A. celosiicola* (as *A. celosiae* = *N. celosiae*), *Alternaria perpunctulata* (= *Nimbya perpunctulata*), and *Alternaria alternantherae* (= *Nimbya alternantherae*)—were phylogenetically close to each other and were confined to amaranthaceous hosts. Our data agreed (Fig. 4.8). Furthermore, host differentiation occurred among the group II sub-clade (Lawrence *et al.* 2012) of former *Nimbya* species; *Alternaria celosiicola* was pathogenic to representatives of *Amaranthoideae* and *Gomphrenoideae* but not to those of *Betoideae* or *Chenopodioideae* (Table 4.1, Fig. 4.3c–h), while according to inoculation tests by Pomella *et al.* (2007), *A. alternantherae* was pathogenic to *Alternanthera philoxeroides* (alligator weed; *Gomphrenoideae*), *C. argentea*, *B. vulgaris*, *S. oleracea*, and *Portulaca halimoides* (silkcotton purslane; *Portulacaceae*), but not to *Alternanthera ficoidea* (sanguinaria; *Gomphrenoideae*), *Amaranthus spinosus* (spiny amaranth; *Amaranthoideae*), *G. globosa*, or *Pfaffia paniculata* (suma; *Gomphrenoideae*). According to Holcomb (1978), *A*. *alternantherae* was strongly pathogenic to *C*. *argentea* and *Iresine harbstii* (bloodleaf; *Gomphrenoideae*), moderately pathogenic to *A*. *philoxeroides* and *Alternanthera bettzickiana* (calico plant; *Gomphrenoideae*), and weakly pathogenic to *Amaranthus* spp. and *G*. *globosa*. Thus, a species concept that integrates morphology, phylogeny, and pathogenicity was effective to delimit this species; however, additional studies on morphology and experimental host ranges are needed to characterize each related former *Nimbya* species on *Amaranthaceae*.

Alternaria crassa

Alternaria crassa resembles *Alternaria solani* Sorauer in morphology but is clearly distinguishable from other members of the "porri sp.-group" by its long, colored false beak and absence of pigment on medium (Ellis 1971). In addition, *A. crassa* differs from *A. solani* in its

lack of pathogenicity to *Solanum tuberosum* (potato; *Solanaceae*) (Rands 1917; Neergaard 1945). Furthermore, *A*. *daturicola* (in Zhang 2003) on *D*. *stramonium* is distinguishable from *A. crassa* by its short and broad conidial bodies and very long beaks (7.5 × the length of the conidial body). However, the isolate of *A. crassa* examined in this study included morphological characteristics of both species, and its ITS sequence was similar to that of a strain identified as *A*. *daturicola*. Therefore, further comparative studies using type material and ex-type strains will be needed to determine whether *A. daturicola* should be treated as a later synonym of *A. crassa*.

The morphological characteristics of *A. capsici* on *C. annuum* and *Alternaria grandis* E.G. Simmons on *S*. *tuberosum* described by Simmons (2000) were similar to those of *A. crassa*. The strong pathogenicity of our isolate of *A. crassa* to *C*. *annuum* (Table 4.1, Fig. 4.5c–g) also suggested that *A. capsici* may be a later synonym of *A. crassa*. Phylogenetic relationships based on *act*, *Alt a 1* and *gapdh* sequences of *A*. *crassa* and *A*. *capsici* (Hong *et al.* 2005b), as well as morphology, also supported this suggestion, although further cross-inoculations with each ex-type strain should be made.

Alternaria grandis could be regarded as a distinctive species not only because *A. crassa* had no pathogenicity on *S. tuberosum* (Neergaard 1945) but also because of its beak morphology and characteristic color. In addition, although *A. crassa* has been isolated from other solanaceous plants, such as *Nicandra physalodes* (shoo-fly plant; *Nicandreae*) (Simmons 2000), *Solanum nigrum* (black nightshade; *Solaneae*) (Zhang 2003), and *Petunia* × *hybrida* (subfamily *Petunioideae*) (Rao 1969), its pathogenicity to *N*. *physalodes* and *S. nigrum* in inoculation tests was doubtful. Chupp (1953) also described other plant taxa as the hosts of several varieties of this species (as *Cercospora crassa*), but some may have been other large-spored *Alternaria* species rather than *A*. *crassa* based on their reported hosts and our inoculation results.

Based on inoculation results and the phylogenetic position of *A. crassa* within the "porri sp.-group" (Fig. 4.8), the species in this group may have host ranges that vary from wide (multiple families, subfamilies, or tribes) to narrow (restricted to a few genera) (Neergaard 1945). In fact, *Alternaria dauci* (J.G. Kühn) J.W. Groves & Skolko was pathogenic not only to *D*.

carota but also to *Anethum graveolens* and *F. vulgare* in inoculation tests (Boedo *et al.* 2012). Likewise, *A. crassa* is not only pathogenic to *Datura* but also to *Capsicum* and other species beyond the tribe *Datureae* (Table 4.1, Fig. 4.5c–d). Given a phylogeny of subfamily *Solanoideae* (Olmstead *et al.* 2008), if pathogenicity tests of each ex-type indicated that it had the same host range as our *A. crassa* isolate, one could conclude that the tribes *Solaneae* and *Physaleae* gained resistance genes to *A. crassa* in the course of evolution. Further inoculation studies guided by plant phylogeny are required to reveal the host range and taxonomy of this group.

Alternaria petroselini

Alternaria petroselini is clearly distinguishable from the related *Alternaria radicina* Meier, Drechsler & E.D. Eddy and *Alternaria carotiincultae* E.G. Simmons in that it is non-pathogenic to *D. carota* (Neergaard 1945; Pryor & Gilbertson 2002; Park *et al.* 2008). Although the natural host range of *A. petroselini* was restricted to tribes *Apieae* and *Coriandreae* (Farr & Rossman 2012), it may have a wide host range within *Apiaceae* given that it infected members of *Bupleureae* as well as *Scandiceae* (Table 4.1, Fig. 4.7h–m).

Another closely related species, *A. selini*, differing from *A. petroselini* in its long-ellipsoid conidium, was described by Simmons (1995); however, the conidia of both species varied in both shape and size during culturing. Our isolate of *A. petroselini* frequently produced long-ellipsoid conidia on V8 and PCA media, and the two species were difficult to distinguish. Moreover, rDNA sequences have never discriminated these two species in previous studies using their ex-type strains (Pryor & Gilbertson 2000; Pryor & Bigelow 2003; Park *et al.* 2008; Runa *et al.* 2009; Lawrence *et al.* 2012), nor were their ITS sequences distinct in the present study (Fig. 4.8). The possible conspecificity of *A. petroselini* and *A. selini* has not been previously discussed. Such taxonomic confusion disturbs plant pathologists; Cunnington *et al.* (2007) identified their Australian isolate on parsley as *A. petroselini sensu lato* because of its morphological variability. The morphological definitions of these two species (Simmons 1995) may be misleading; we suggest that *A. selini* is probably a later synonym of *A. petroselini*.

Further studies on the morphology, phylogeny, and pathology of type material are needed to synonymize the taxa.

In this paper, we characterized four species of *Alternaria* to establish a species concept that integrated morphology, phylogeny, and pathology and suggested that experimental host range would correlate with morphological taxonomy and phylogenetic relationships. Describing species with this concept, especially incorporating pathology, will certainly help plant pathologists diagnose and control diseases caused by *Alternaria*. Although phylogenetic analysis of the ITS region in *Alternaria* and its allies may raise objections, the focus of this integrated species concept is not fungal systematics but species delimitation; when integrated with morphological and pathogenicity data, ITS sequence data can provide taxonomic insights. In future studies, we plan to address phylogenetic relationships among Japanese species of *Alternaria* and allied genera using our existing sequence data for *Alt a 1*, *gapdh*, and *act*, as well as additional sequences of *EF-1α*, *endoPG*, *BT1*, *BT2*, and *rpb2*.

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Chapter 5

Japanese species of *Alternaria* and their species boundaries based on host range

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ABSTRACT

To clarify the diversity of plant-parasitic *Alternaria* species in Japan, diseased leaves were collected and isolates of the genus were identified. We examined 85 isolates consisting of 23 species covering 14 known phylogenetic sections (including former *Crivellia*, *Nimbya*, and *Ulocladium* species, based on their conidial morphology and phylogenetic analysis), as well as three new species. To clarify the species boundaries of some of the collected species, phenotypes with experimental host ranges were also determined. *Alternaria gomphrenae* and the related species in sect. *Alternantherae*, including the novel species ex *Gomphrena*, were recognizable as distinct species owing to their host selectivity. The experimental host ranges of three morphologically and phylogenetically distinct species infecting *Brassicaceae* plants (*A. brassicae*, *A. brassicicola*, and *A. japonica*) showed almost no differences; however, some gaps between these three species were found on *Eutrema*. Among the species infecting *Apiaceae*, the pathogenicity of *A. cumini* (a newly recorded species from Japan) and a novel species ex *Bupleurum* were strictly selective to each original host. Ex *Petunia*, another novel species, also demonstrated its host selectivity on solanaceous plants. *Alternaria iridicola* was recognized as a large-spored species in sect. *Alternaria*, having long false beaks and host species selectivity toward *Iris* spp. On the other hand, distinctive pathogenic species *A. brassicicola* and *A. porri* were also found on non-host plants. It was concluded that the host ranges of *Alternaria* species reflected species recognition based on morphology and molecular phylogeny, and that phenotyping combined with determination of host range makes species boundaries within the genus clearer and more practical. Based on integrated species recognition, synonymizable taxa were also determined for *A. brassicicola* and *A. japonica*. This study also contributes to the designation of a lectotype for *A. gomphrenae*, and to the epitypes of *A. cinerariae*, *A. gomphrenae*, *A. iridicola*, and *A. japonica*.

Keywords: Host range, Morphology, Phenotyping, Phylogeny, Taxonomy

INTRODUCTION

Alternaria Nees is an anamorphic genus in the phylum *Ascomycota* (*Pleosporaceae*, *Pleosporales*) characterized by phaeodictyospores or phaeophragmospores (Seifert *et al.* 2011), and is generally one of the most ubiquitous fungal genera, inhabiting every environmental substrate (atmosphere, soil, litter, and living plants) (Guo *et al.* 2004; Kirk *et al.* 2008). They are often allergenic, and can cause mycoses in humans and insects (Rossmann *et al.* 1996; Christias *et al.* 2001; Downs *et al.* 2001); however, most species are plant pathogenic (Yu 2001). *Alternaria* species usually cause leaf spot diseases, especially for vegetables and ornamental flowers; however, it is their seed-borne phases that carry the greatest economic importance (Groves & Skolko 1944; Neergaard 1945; Richardson 1990; Tohyama 1993; Rathod 2012).

Alternaria was established and originally typified by *Alternaria tenuis* Nees, and was redefined as a genus related to *Stemphylium* and *Ulocladium* based on their mode of conidiogenesis (Simmons 1967). Two additional genera, *Embellisia* and *Nimbya*, subsequently established by Simmons (1971, 1989), had also been recognized as related genera until recently. The taxonomy of *Alternaria* and its allied genera was based previously on conidial morphology, sporulation patterns, and differences in their host plants (mostly at the rank of genus) or substrates as species keys (Simmons 2007). However, their morphological variation and fundamental pleomorphism made species recognition complicated, and the use of host plants as a taxonomic key (which may include false hosts because of their ubiquitousness) resulted in the genus being split into more than 400 species (Nishikawa & Nakashima 2015; Lawrence *et al.* 2016). The latest molecular phylogenies have not only helped to clarify the taxonomy, but have also combined the above allied genera into one large genus, *Alternaria* (Woudenberg *et al.* 2013).

Despite the application of molecular phylogenetic analyses, the relationships between taxonomy and plant parasitism remain insufficient to aid the practical recognition of species boundaries, and additional characterization is needed. Therefore, we proposed integrated species recognition based on morphology, molecular phylogeny, and pathogenicity (Nishikawa

& Nakashima 2013). In our previous studies, it was suggested that phenotyping combined with the determination of experimental host range was very helpful to find potential susceptible hosts and possible synonyms of examined species (Nishikawa & Nakashima 2013, 2015).

During the survey of Japanese species of *Alternaria*, we have collected and examined 85 isolates, and the integrated species recognition method was extended to Japanese species. The present study focused on biodiversity and the utility of phenotyping based on systematic experimental host ranges determined by inoculation tests. In addition, morphological observation and phylogenetic analyses were conducted to distinguish closely related species infecting *Amaranthaceae*, *Apiaceae*, *Brassicaceae*, *Iridaceae*, and *Solanaceae*.

MATERIAL AND METHODS

Fungal collection and isolation

The 85 isolates examined in the present study were obtained from diseased leaves, stems, buds, rhizomes, and seeds of various plants on the basis of field surveys in Japan from 2002 to 2018 (Table 5.1). Some of the isolates and specimens were obtained from the culture collection at the Genetic Resources Center of the National Agriculture and Food Research Organization (NARO; MAFF), Tsukuba, Japan, and several collaborators who assisted the acquisition of these specimens are mentioned in the acknowledgements. To establish purified cultures originating from single conidia, alternarioid conidia from lesions were suspended in sterilized distilled water and spread on 2 % aqueous agar (WA) medium using a flame-sterilized microspatula. After incubation at 20 °C for 24 h, individual germinating conidia were transferred to potato-carrot agar (PCA; Simmons 2007) using a flame-sterilized microtube under a light microscope at a magnification of ×100 (Nakashima *et al.* 2011). Deposits of the representative isolates from the present study were made to NARO and Mie University (MUCC), Tsu, Mie, Japan. Specimens, including holotype and epitype were also deposited in TNS (National Museum of Nature and Science), Tsukuba, Ibaraki, Japan, and/or in TSU (Mie University).

Table 5.1. Isolates of Japanese species of *Alternaria* obtained in this study.

Fungal name	*Alternaria* section	Strain number[1,2]	Host plant	Location; year
Alternaria alstroemeriae	*Alternaria*			
		MAFF 241374 = AC46	*Alstroemeria* sp.	Nagano Pref., Matsumoto; 2008
Alternaria alternata	*Alternaria*			
		MAFF 239887 = AC48	*Vigna radiata*	unknown (Japan); 1998
		MUCC 1610 = AC51	*Impatiens hawkeri*	Nagano Pref., Azumino; 2006
		MUCC 1611 = AC54	*Antirrhinum majus*	Shizuoka Pref., Kakegawa; 2008
		MUCC 1616 = AC66	*Pelargonium hortorum*	Kanagawa Pref. Nakai; 2004
		MUCC 1617 = AC67	*Primula × polyantha*	Shizuoka Pref., Kakegawa; 2004
		AC82	*Solanum lycopersicum*	Shizuoka Pref., Kakegawa; 2011
		MAFF 243775 = AC104	*Vigna radiata*	Tokyo, Chiyoda; 2012
		MAFF 305014	*Pyrus aromatica*	Kanagawa Pref.; 1958
		MAFF 410775	unknown (*Pyrus*?)	unknown (Japan)
Alternaria atra	*Ulocladioides*			
		AC86	*Raphanus sativus*	Tokyo, Setagaya; 2000
		AC87	*Brassica oleracea* var. *capitata*	Tokyo, Setagaya; 2001
		AC88	*Brassica rapa* subsp. *pekinensis*	Tokyo, Setagaya; 2001
		MAFF 246889 = AC90	*Allium fistulosum*	Tokyo, Setagaya; 2001
Alternaria botrytis	*Ulocladium*			
		MAFF 246887 = AC52	*Asparagus officinalis*	Shizuoka Pref., Kakegawa; 2008
Alternaria brassicae				
		AC29	*Brassica rapa*	Shizuoka Pref., Kakegawa; 2006
		MAFF 240791 = AC47	*Raphanus sativus*	Ibaraki Pref., Tsukuba; 2007
		MUCC 1615 = AC62	*Raphanus sativus*	Chiba Pref. Narita; 2009

Table 5.1. (Continued).

Fungal name	*Alternaria* section	Strain number[1,2]	Host plant	Location; year
Alternaria brassicicola	*Brassicicola*			
		MAFF 246772 = MUCC 1694 = AC23	*Brassica oleracea* var. *sabellica*	Shizuoka Pref., Kakegawa; 2003
		MAFF 246773 = AC26	*Spinacia oleracea*	Tokyo, Setagaya; 2002
		MUCC 1612 = AC56	*Brassica rapa* var. *glabra*	Shizuoka Pref., Kakegawa; 2008
		MUCC 1619 = AC70	*Raphanus sativus*	Tokyo, Setagaya; 2000
		AC71	*Raphanus sativus*	Tokyo, Setagaya; 2000
		AC72	*Brassica oleracea* var. *italica*	Tokyo, Setagaya; 2001
Alternaria celosiicola	*Alternantherae*			
		MAFF 243058 = AC28	*Celosia argentea* var. *plumosa*	Kanagawa Pref., Fujisawa; 2006
Alternaria chartarum	*Pseudoulocladium*			
		MAFF 246888 = AC85	*Capsicum annuum*	Tokyo, Setagaya; 2000
Alternaria cinerariae	*Sonchi*			
		MAFF 243059 = MUCC 1701 = AC3^{ET}	*Pericallis cruenta*	Chiba Pref., Narita; 2002
		MAFF 241266 = MUCC 1613 = AC57	*Farfugium japonicum*	Ibaraki Pref., Tsukuba; 2008
		MAFF 241267 = MUCC 1614 = AC58	*Gynura bicolor*	Ibaraki Pref., Tsukuba; 2008
		MUCC 2504 = AC138	*Jacobaea maritima*	Kanagawa Pref., Atsugi; 2017
Alternaria crassa	*Porri*			
		MAFF 243056 = AC4	*Datura stramonium*	Tokyo, Kodaira; 2000
		MUCC 2502 = AC131	*Datura fastuosa*	Tokyo, Kodaira; 2012
		MUCC 2503 = AC132	*Datura inoxia*	Tokyo, Kodaira; 2012
Alternaria cucumerina	*Porri*			
		AC105	*Cucurbita maxima*	Niigata Pref., Sado; 2010
		AC106	*Cucurbita maxima*	Niigata Pref., Sado; 2010

Table 5.1. (Continued).

Fungal name	*Alternaria* section	Strain number[1,2]	Host plant	Location; year
Alternaria cumini	*Eureka*			
		MAFF 246774 = AC94	*Cuminum cyminum*	Shizuoka Pref., Kakegawa; 2012
		AC115	*Cuminum cyminum*	Shizuoka Pref., Kakegawa; 2013
Alternaria dauci	*Porri*			
		MUCC 1684 = AC8	*Daucus carota*	Shizuoka Pref., Kakegawa; 1998
		AC9	*Daucus carota*	Shizuoka Pref., Kakegawa; 1998
Alternaria gaisen f. sp. *fragariae*	*Alternaria*			
		MAFF 242310 = MUCC 1609 = AC49	*Fragaria* × *ananassa* 'HS-138'	Hokkaido, Esahi; 2007
		MAFF 731001	*Fragaria* × *ananassa* 'Morioka-16'	Iwate Pref., Morioka; 1975
		MAFF 731002	*Fragaria* × *ananassa* 'Morioka-16'	Iwate Pref., Morioka; 1975
		MAFF 731003	*Fragaria* × *ananassa* 'Morioka-16'	Iwate Pref., Morioka; 1975
		MAFF 731004	*Fragaria* × *ananassa* 'Morioka-16'	Iwate Pref., Morioka; 1975
		MAFF 731005	*Fragaria* × *ananassa* 'Morioka-16'	Iwate Pref., Morioka; 1975
		MAFF 731006	*Fragaria* × *ananassa* 'Morioka-16'	Iwate Pref., Morioka; 1975
		MAFF 731007	*Fragaria* × *ananassa* 'Morioka-16'	Iwate Pref., Morioka; 1975
Alternaria gaisen f. sp. *pyri*	*Alternaria*			
		MUCC 2151 = AC116 = 9901A	*Pyrus pyrifolia* var. *culta* 'Nijisseiki'	Tottori Pref., Tohaku; 1999
		MUCC 2152 = AC117 = 9903A	*Pyrus pyrifolia* var. *culta* 'Nijisseiki'	Tottori Pref., Tohaku; 1999
		MUCC 2153 = AC118 = 9904C	*Pyrus pyrifolia* var. *culta* 'Nijisseiki'	Tottori Pref., Tohaku; 1999
Alternaria gomphrenae	*Alternantherae*			
		MAFF 246769 = MUCC 1623 = AC81[ET]	*Gomphrena globosa*	Shizuoka Pref., Kakegawa; 2011
Alternaria iridicola	*Alternaria*			
		MUCC 2148 = AC113	*Iris japonica*	Tokyo, Kodaira; 2010

Table 5.1. (Continued).

Fungal name	*Alternaria* section	Strain number[1,2]	Host plant	Location; year
		MAFF 246890 = MUCC 2149 =AC114[ET]	*Iris japonica*	Kanagawa Pref., Kamakura; 2013
		MAFF 246771 = MUCC 2501 = AC139	*Iris japonica*	Shizuoka Pref., Fukuroi; 2018
Alternaria japonica	*Japonicae*			
		AC73	*Raphanus sativus*	Tokyo, Setagaya; 2000
		MAFF 246775 = MUCC 1622 = AC74[ET]	*Raphanus sativus*	Tokyo, Setagaya; 2000
		AC96	*Brassica oleracea* var. *italica*	Shizuoka Pref., Kakegawa; 2010
		AC97	*Brassica oleracea* var. *italica*	Shizuoka Pref., Kakegawa; 2010
Alternaria nobilis	*Gypsophilae*			
		AC1	*Dianthus barbatus*	Shizuoka Pref., Kakegawa; 2003
		AC25	*Dianthus caryophyllus*	Miyagi Pref., Sendai; 2002
Alternaria panax	*Panax*			
		MUCC 1692 = AC18 = PFAlt1-1	*Polyscias fruticosa*	Tokyo, Ogasawara (Bonin Is.); 2003
		AC19 = PGAlt1	*Polyscias guilfoylei*	Tokyo, Ogasawara (Bonin Is.); 2003
		MAFF 243161 = MUCC 1625 = AC83	*Polyscias fruticosa*	Tokyo, Ogasawara (Bonin Is.); 2011
		MAFF 243162 = MUCC 1626 = AC84	*Polyscias fruticosa*	Tokyo, Ogasawara (Bonin Is.); 2011
Alternaria penicillata	*Crivellia*			
		MUCC 1657 = AC102	*Papaver nudicaule*	Tokyo, Tachikawa; 2005
Alternaria petroselini	*Radicina*			
		MAFF 243057 = AC42	*Petroselinum crispum*	Shizuoka Pref., Kakegawa; 2007
Alternaria porri	*Porri*			
		AC2	*Viola × wittrockiana*	Shizuoka Pref., Kakegawa; 2003
		AC6	*Calibrachoa* sp.	Shizuoka Pref., Kakegawa; 2004
		MUCC 1688 = AC14	*Allium fistulosum*	Shizuoka Pref., Kakegawa; 2004

Table 5.1. (Continued).

Fungal name	*Alternaria* section	Strain number[1,2]	Host plant	Location; year
		AC15	*Allium fistulosum*	Saitama Pref.; 2004
		AC16	*Allium fistulosum*	Gunma Pref., Takasaki; 2005
		AC17	*Allium fistulosum*	Gunma Pref., Takasaki; 2005
		MUCC 1698 = AC30	*Allium fistulosum*	Gunma Pref., Tomioka; 2006
		AC32	*Allium fistulosum*	Chiba Pref., Mobara; 2006
		MUCC 1702 = AC35	*Eustoma exaltatum* subsp. *russellianum*	Shizuoka Pref., Kakegawa; 2007
		AC68	*Allium fistulosum*	Tokyo, Setagaya; 2001
Alternaria zinniae	*Porri*			
		MUCC 1704 =AC44	*Zinnia hybrida*	Nagano Pref., Tomi; 2007
		AC107	*Zinnia hybrida*	Nagano Pref., Azumino; 2010
		AC108	*Zinnia elegans*	Shizuoka Pref., Kakegawa; 2011
		AC109	*Zinnia elegans*	Nagano Pref., Azumino; 2011
Alternaria sp.				
	Alternaria	MAFF 305015	*Pyrus aromatica*	Chiba Pref.; 1959
Novel species				
	Alternantherae	MAFF 246768 = AC7^T	*Gomphrena haageana*	Shizuoka Pref., Hamamatsu; 2004
	Alternaria	MAFF 246770 = AC34^T	*Petunia* × *atkinsiana*	Shizuoka Pref., Kakegawa; 2006
	not assigned	MAFF 246776 = AC5^T	*Bupleurum rotundifolium*	Kochi Pref., Konan; 2004
		AC95	*Bupleurum rotundifolium*	Shizuoka Pref., Kakegawa; 2004

[1] AC: Personal collection of JN; MAFF: Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Japan; MUCC (Japan): Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Japan.

[2] Ex-type and -epitype strain indicated with T and ET, respectively.

Morphological observation and culture characteristics

For microscopic observations of diagnostic morphology comparable to those of Simmons's standard conditions (2007), sporulation was induced according to methods reported by Nishikawa & Nakashima (2013). After incubation of the isolates at 25 °C in the dark for 7 d on PCA and V8 juice agar (V8; Simmons 2007), the growing colonies were scratched with a flame-sterilized microspatula and the aerial mycelia were removed to observe sporulation more easily. Treated colonies in unsealed Petri dishes were incubated for 12–24 h at 25 °C under blacklight blue fluorescent lamps to induce sporulation, and then the plates were transferred to at 20 °C in the dark. Caespituli that formed on the medium 7 d after incubation were mounted with Shear's mounting fluid [300 mL aqueous potassium acetate (2 %), 120 mL glycerin, and 180 mL ethanol (95 %)]. The morphology of 100 conidia and other structures, such as conidiophores and chlamydospores, were examined at a magnification of ×400, and sporulation patterns were also observed under a light microscope.

Mycelial discs of each isolate were plated onto potato-dextrose agar (PDA; 40 g potato, 20 g dextrose, and 20 g agar in 1.0 L distilled water) plates. The diameter of each of five colonies was measured after incubation in the dark for 7 d at 25 °C, and the mean diameters for a species were calculated with 95 % confidence intervals. Culture characteristics were also recorded.

To induce sexual reproduction in our collected species, we applied the rice straw agar (RSA) method reported by Tanaka & Harada (2003). Rice straws 4–5 cm long were soaked in distilled water in a glass vial, autoclaved, and then three pieces of each straw were plated on WA. Mycelial discs of each isolate were plated and pre-incubated at 20 °C in the dark for 2 wks. To induce the production of ascomata, the plates were transferred and incubated under blacklight blue fluorescent lamp irradiation for three months.

DNA extraction and phylogenetic analyses

An UltraClean Microbial DNA isolation kit (MoBio Laboratories, Carlsbad) was used to conduct DNA extraction. PCR amplification and sequencing of the rDNA internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), RNA polymerase second largest

subunit (*rpb2*), translation elongation factor 1-alpha (*tef1*), Alternaria major allergen (*Alt a 1*), and endopolygalacturonase (*endoPG*) were conducted at the Mie University Advanced Science Research Promotion Center, according to the procedure described in previous studies (Nishikawa & Nakashima 2013, 2015, 2019). All the newly determined sequences were deposited in the DNA Data Bank of Japan (DDBJ) (Table 5.2). Complementary strands of the sequences were assembled and concatenated in MEGA v. 7 (Kumar *et al.* 2016) and were aligned using MAFFT v. 7 (Katoh *et al.* 2017; http://mafft.cbrc.jp/alignment/server/index.html).

To analyze the relationships between Japanese isolates and existing species, maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses were conducted using a combined dataset composed of 80 *gapdh*, *rpb2*, and *tef1* sequences generated from our collected Japanese isolates and other sequences from GenBank (Table 5.2). MP analyses were performed in PAUP* v. 4.0b10 (Swofford 2003) using heuristic searches, each of which consisted of 100 random sequence additions and a tree-bisection-reconnection (TBR) algorithm for branch swapping. All the characters were unordered and unweighted, with alignment gaps treated as missing data. Clade robustness of the obtained trees was assessed using 1000 bootstrap (BS) replications (Felsenstein 1985). Tree scores, including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI), were calculated. ML analyses were performed in RAxML-NG v. 0.6.0 BETA (Kozlov *et al.* 2018) using the GTR+FO+G model as the nucleotide substitution model and 100 BS replicates. BI analyses were performed in BEAST v. 2.5.1 (Bouckaert *et al.* 2014). A nucleotide substitution model TN93 was selected by Kakusan4 software (Tanabe 2011). To estimate the posterior probabilities (PPs) of tree topologies, Metropolis-Coupled Markov Chain Monte Carlo searches (MCMCMC) were run for 30 million generations with trees sampled and saved every 1000 generations, which generated 18,001 trees from which the initial 12,000 trees were discarded as burn-in. After discarding, PPs were determined from the remaining trees. Sequences of *Paradendryphiella salina* (= *E. annulata*) (CBS 302.84) were used as the outgroup.

Table 5.2. Isolates and their accession numbers for phylogenetic analyses.

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	DDBJ/GenBank/EMBL accession numbers[3]						
			ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
Alternantherae	*Alternaria alternantherae* (= *Nimbya alternantherae*)								
	EGS52.039	unknown, *Alternanthera philoxeroides*?	JN383496	JN383477	JQ672485	–	JN383511	–	JQ671717
	CBS 124392; HSAUP2798	China, *Solanum melongena*	KC584179	KC584096	KC584633	KC584374	KP123846	–	–
	***A. celosiicola* (= *A. cristata*)**								
	MAFF 243058; AC28	**Japan, *Celosia argentea* var. *plumosa***	AB678217	AB744033	*	*	AB744029	–	AB744036
	EGS42.013^{T}	USA, *Celosia cristata*	JN383497	JN383478	JQ672483	JQ646495	JN383512	–	JQ671716
	A. gomphrenae (= *N. gomphrenae*)								
	MAFF 246769; MUCC 1623; AC81^{ET}	**Japan, *Gomphrena globosa***	**LC440579**	*	*	*	*	–	*
	A. perpunctulata (= *N. perpunctulata*)								
	CBS 115267; EGS51.130^{T}	USA, *Alternanthera philoxeroides*	KC584210	KC584129	KC584676	KC584418	JQ905111	–	JQ671718
	***Alternaria* sp.**								
	MAFF 246768; AC7^{T}	**Japan, *Gomphrena haageana***	–	*	*	*	*	–	*
Alternaria	*A. alstroemeriae*								
	MAFF 241374; AC46	**Japan, *Alstroemeria* sp.**	AB678214	AB744034	LC275050	LC275231	AB744031	LC276240	AB744038
	CBS 118809; EGS52.068^{T}	Australia, *Alstroemeria* sp.	KP124297	KP124154	KP125072	KP124765	–	KP123994	–
	A. alternata								
	MAFF 239887; AC48	**unknown, *Vigna radiata***	**LC440580**	*	*	*	*	*	*
	MUCC 1610; AC51	**Japan, *Impatiens hawkeri***	LC269968	LC270135	LC275052	LC275233	LC276230	LC276242	*
	MUCC 1611; AC54	**Japan, *Antirrhinum majus***	**LC440581**	LC270134	LC275051	LC275232	LC276229	LC276241	*
	MUCC 1616; AC66	**Japan, *Pelargonium hortorum***	LC269969	LC270136	LC275053	LC275234	LC276231	LC276243	*
	MUCC 1617; AC67	**Japan, *Primula polyantha***	**LC440582**	*	–	*	*	*	*
	AC82	**Japan, *Solanum lycopersicum***	**LC440583**	*	*	*	*	*	*

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	MAFF 243775; AC104	**Japan, *Vigna radiata***	**LC164855**	**LC169124**	**LC167147**	*	**LC167084**	–	–
	MAFF 305014	**Japan, *Pyrus aromatica***	**LC164847**	*	**LC167153**	*	*	*	–
	MAFF 410775	**Japan, *Pyrus*?**	**LC164846**	*	**LC167155**	*	**LC167089**	*	–
	CBS 916.96; EGS34.016[ET]	India, *Arachis hypogaea*	AF347031	AY278808	KC584634	KC584375	AY563301	JQ811978	JQ671702
	CBS 918.96; EGS34.015 (= *A. tenuissima*[R])	UK, *Dianthus chinensis*	AF347032	AY278809	KC584693	KC584435	AY563302	KP124026	JQ671703
	CBS 121348; EGS50.070 (= *A. platycodonis*[T])	China, *Platycodon grandiflorus*	KP124367	KP124219	KP125144	KP124836	KP123915	KP124070	–
	CBS 101.26 (as *A. iridis*)	unknown	–	JQ646313	JQ672475	JQ646482	JQ646396	–	JQ671694
	A. alternata f. sp. *citri* pathotype rough lemon (= *A. limoniasperae*[T])								
	CBS 102595; EGS45.100; BMP0316	USA, *Citrus jambhiri*	FJ266476	AY562411	KC584666	KC584408	AY563306	KP124029	JQ671704
	A. alternata f. sp. *citri* pathotype tangerine (= *A. toxicogenica*[T])								
	CBS 102600; EGS39.181; ATCC 38963	USA, *Citrus reticulata*	KP124331	KP124186	KP125107	KP124799	KP123880	KP124033	–
	A. alternata f. sp. *mali* (= *A. mali*[T])								
	CBS 106.24; EGS38.029; ATCC 13963	USA, *Malus sylvestris*	KP124298	KP124155	KP125073	KP124766	KP123847	AY295020	–
	A. arborescens species complex								
	CBS 102605; EGS39.128; BMP0308[T] (= *A. alternata* tomato pathotype)	USA, *Solanum lycopersicum*	AF347033	AY278810	KC584636	KC584377	AY563303	AY295028	JQ671705
	CBS 119544; EGS43.072 (= *A. cerealis*[T])	New Zealand, *Avena sativa*	KP124408	JQ646321	KP125186	KP124878	KP123955	KP124112	JQ671708
	CBS 124283	Russia, *Oryza* sp.	KP124416	KP124267	KP125194	KP124885	KP123963	KP124120	–
	CPC 25266	Austria, *Pyrus* sp.	KP124418	KP124269	KP125196	KP124887	KP123965	KP124122	–
	A. betae-kenyensis								
	CBS 118810; EGS49.159[T]	Kenya, *Beta vulgaris* var. *cicla*	KP124419	KP124270	KP125197	KP124888	KP123966	KP124123	–
	A. burnsii								
	CBS 107.38; EGS06.185[T]	India, *Cuminum cyminum*	KP124420	JQ646305	KP125198	KP124889	KP123967	KP124124	JQ671685

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	A. eichhorniae								
	CBS 489.92; ATCC 22255[T]	India, *Eichhornia crassipes*	KC146356	KP124276	KP125204	KP124895	KP123973	KP124130	–
	A. gaisen f. sp. *fragariae* (= *A. alternata* strawberry pathotype)								
	MAFF 731001	**Japan, *Fragaria* × *ananassa* 'Morioka-16'**	LC164854	LC169125	LC167148	LC169131	LC276235	LC276246	–
	MAFF 731002	**Japan, *Fragaria* × *ananassa* 'Morioka-16'**	LC164853	LC169126	LC167149	LC169132	LC276236	–	–
	MAFF 731003	**Japan, *Fragaria* × *ananassa* 'Morioka-16'**	LC164852	LC169127	LC167150	LC169133	LC167085	LC276247	–
	MAFF 731004	**Japan, *Fragaria* × *ananassa* 'Morioka-16'**	LC164851	LC270140	LC167151	LC275238	–	LC276248	–
	MAFF 731005	**Japan, *Fragaria* × *ananassa* 'Morioka-16'**	LC164850	LC169128	LC167152	LC169134	LC167086	LC276249	–
	MAFF 731006	**Japan, *Fragaria* × *ananassa* 'Morioka-16'**	LC164849	LC169129	LC275057	LC169135	LC167087	LC276250	–
	MAFF 731007	**Japan, *Fragaria* × *ananassa* 'Morioka-16'**	LC164848	LC169130	LC275058	LC169136	LC167088	LC276251	–
	MAFF 242310; MUCC 1609; AC49	**Japan, *Fragaria* × *ananassa* 'HS-138'**	LC269973	LC270141	LC275059	LC275239	LC276237	LC276252	*
	A. gaisen f. sp. *pyri* (= *A. alternata* Japanese pear pathotype)								
	CBS 118488; EGS90.0391[ET]	Japan, *Pyrus pyrifolia* var. *culta* 'Nijisseiki'	KP124427	KP124278	KP125206	KP124897	KP123975	KP124132	–
	CBS 632.93; EGS90.0512[R]	Japan, *Pyrus pyrifolia* var. *culta* 'Nijisseiki'	KC584197	KC584116	KC584658	KC584399	KP123974	AY295033	–
	MUCC 2151; AC116; 9901A	**Japan, *Pyrus pyrifolia* var. *culta* 'Nijisseiki'**	LC269970	LC270137	LC275054	LC275235	LC276232	–	–
	MUCC 2152; AC117; 9903A	**Japan, *Pyrus pyrifolia* var. *culta* 'Nijisseiki'**	LC269971	LC270138	LC275055	LC275236	LC276233	LC276244	–
	MUCC 2153; AC118; 9904C	**Japan, *Pyrus pyrifolia* var. *culta* 'Nijisseiki'**	LC269972	LC270139	LC275056	LC275237	LC276234	LC276245	–
	A. gossypina								
	CBS 104.32[T]	Zimbabwe, *Gossypium* sp.	KP124430	JQ646312	KP125209	KP124900	JQ646395	KP124135	JQ671693
	A. iridiaustralis								
	CBS 118486[T]; EGS43.014	Australia, *Iris* sp.	KP124435	KP124284	KP125214	KP124905	KP123981	KP124140	–
	A. iridicola								
	MUCC 2148; AC113	**Japan, *Iris japonica***	LC269974	LC270142	LC275060	LC275240	LC276238	LC276253	–

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	MAFF 246890; MUCC 2149; AC114[ET]	**Japan, *Iris japonica***	LC269975	LC270143	LC275061	LC275241	LC276239	LC276254	–
	MAFF 246771; MUCC 2501; AC139	**Japan, *Iris japonica***	–	–	*	*	*	–	*
	A. jacinthicola								
	CBS 133751[T]	Mali, *Eichhornia crassipes*	KP124438	KP124287	KP125217	KP124908	KP123984	KP124143	–
	A. longipes (= *A. alternata* tobacco pathotype)								
	CBS 540.94; EGS30.033[R]	USA, *Nicotiana tabacum*	AY278835	AY278811	KC584667	KC584409	AY563304	KP124147	JQ671689
	CBS 121332; EGS30.048[R]	USA, *Nicotiana tabacum*	KP124443	KP124292	KP125222	KP124913	KP123989	KP124149	–
	A. tomato								
	CBS 114.35	Unknown, *Solanum lycopersicum*	KP124446	KP124295	KP125225	KP124916	KP123992	KP124152	JQ671686
	***Alternaria* sp.**								
	MAFF 246770; AC34[T]	**Japan, *Petunia × atkinsiana***	**LC440584**	*	*	*	*	*	*
Brassicicola	*A. brassicicola*								
	MAFF 246772; MUCC 1694; AC23	**Japan, *Brassica oleracea* var. *sabellica***	**LC440585**	*	*	*	*	–	*
	MAFF 246773; AC26	**Japan, *Spinacia oleracea***	–	*	*	*	*	–	*
	MUCC 1612; AC56	**Japan, *Brassica rapa* var. *glabra***	**LC440586**	AB862969	AB862981	AB862975	*	–	*
	MUCC 1619; AC70	**Japan, *Raphanus sativus***	**LC440587**	AB862968	AB862980	AB862974	*	–	*
	AC71	**Japan, *Raphanus sativus***	**LC440588**	*	*	*	*	–	*
	CBS 118699; EGS42.002; ATCC 96836[R]	USA, *Brassica oleracea*	JX499031	KC584103	KC584642	KC584383	–	–	–
	A. conoidea (= *Embellisia conoidea*)								
	CBS 132.89	Saudi Arabia, *Ricinus communis*	FJ348226	FJ348227	KC584711	KC584452	FJ348228	–	JQ671667
	A. mimicula								
	CBS 118696; EGS01.056; BMP0324[T]	USA, *Solanum lycopersicum*	FJ266477	AY562415	KC584669	KC584411	AY563310	–	JQ671668

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	A. septorioides								
	CBS 106.41; EGS52.089[T]	Netherlands, *Reseda odorata*	KC584216	KC584136	KC584685	KC584427	–	–	–
	A. solidaccana								
	CBS 118698; EGS36.158[T]	Bangladesh, soil	KC584219	KC584141	KC584690	KC584432	–	–	–
Chalastospora	*A. cetera* (= *Chalastospora cetera*)								
	CBS 121340; CBS 110898; EGS41.072; BMP0033[T]	Australia, *Elymus scabrus*	JN383482	AY562398	KC584699	KC584441	AY563278	–	JQ671626
Cheiranthus	*A. cheiranthi*								
	CBS 109384; EGS41.188; BMP0148; BMP0148[R]	Italy, *Cheiranthus cheiri*	AF229457	KC584107	KC584646	KC584387	JQ905106	–	JQ671656
Crivellia	*A. papavericola* (= *Crivellia homothallica, Brachycladium papaveris*)								
	CBS 116606; P351[T]	USA, *Papaver somniferum*	FJ357310	FJ357298	KC584705	KC584446	JN383501	–	JQ671608
	A. penicillata (= *Cr. papaveracea, B. penicillatum*)								
	MUCC 1657; AC102	**Japan, *Papaver nudicaule***	**LC440589**	*	*	*	*	–	*
	CBS 116608; P354.8[ET]	Austria, *Papaver rhoeas*	FJ357311	FJ357299	KC584698	KC584440	JN383502	–	JQ671609
	CBS 116607; P354.1	Austria, *Papaver rhoeas*	KC584229	KC584153	KC584706	KC584447	–	–	–
Dianthicola	*A. dianthicola*								
	CBS 116491; EGS51.022[R]	New Zealand, *Dianthus* × *allwoodii*	KC584194	KC584113	KC584653	KC584394	–	–	–
Embellisia	*A. embellisia* (= *E. allii*)								
	CBS 339.71[R]	USA, *Allium sativum*	KC584230	KC584155	KC584708	KC584449	–	–	–
Embellisioides	*A. hyacinthi* (= *E. hyacinthi*)								
	CBS 416.71; EGS19.102[T]	Netherlands, *Hyacinthus orientalis*	KC584233	KC584158	KC584716	KC584457	–	–	–
Euphorbiicola	*A. euphorbiicola*								
	CBS 119410; EGS41.029[R]	USA, *Euphorbia pulcherrima*	KJ718173	KJ718018	KJ718521	KJ718346	–	–	–

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
Eureka	*A. cumini*								
	MAFF 246774; AC94	**Japan, *Cuminum cyminum***	**LC440590**	*	*	*	*	–	*
	AC115	**Japan, *Cuminum cyminum***	**LC440591**	*	*	*	*	*	–
	CBS 121329; EGS04.1581[T]	India, *Cuminum cyminum*	KC584191	KC584110	KC584650	KC584391	–	–	–
	A. eureka (= *E. eureka*)								
	CBS 193.86; EGS36.103[T]	Australia, *Medicago rugosa*	JN383490	JN383471	KC584715	KC584456	JN383507	–	JQ671596
Gypsophilae	*A. ellipsoidea*								
	CBS 119674; EGS49.104[T]	USA, *Dianthus barbatus*	KC584196	KC584115	KC584655	KC584396	–	–	–
	A. gypsophilae								
	CBS 107.41; EGS07.025[T]	unknown, *Gypsophila elegans*	KC584199	KC584118	KC584660	KC584401	KJ718688	–	JQ671682
	A. nobilis								
	AC1	**Japan, *Dianthus barbatus***	**LC440592**	*	*	*	*	*	*
	AC25	**Japan, *Dianthus caryophyllus***	**LC440593**	*	*	*	*	–	*
	CBS 116490; EGS51.027[R]	New Zealand, *Dianthus caryophyllus*	KC584208	KC584127	KC584673	KC584415	JQ646385	–	JQ671680
	A. saponariae								
	CBS 116492; EGS49.199[R]	USA, *Saponaria officinalis*	KC584215	KC584135	KC584683	KC584425	–	–	–
	A. vaccariicola								
	CBS 118714; EGS46.003[T]	USA, *Vaccaria hispanica*	KC584224	KC584147	KC584697	KC584439	JQ646384	–	JQ671679
Infectoriae	*A. infectoria*								
	CBS 210.86; EGS27.193[T]	UK, *Triticum aestivum*	AF347034	AY278793	KC584662	KC584404	FJ266502	–	JQ671629
Japonicae	*A. japonica*								
	AC73	**Japan, *Raphanus sativus***	**LC440594**	*	*	*	*	*	*
	MAFF 246775; AC74[ET]	**Japan, *Raphanus sativus***	**LC440595**	*	*	*	*	–	*

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	AC96	**Japan, *Brassica oleracea* var. *italica***	**LC440596**	*	*	*	*	–	*
	AC97	**Japan, *Brassica oleracea* var. *italica***	**LC440597**	*	*	*	*	–	*
	CBS 118390; EGS50.099[R]	USA, *Brassica chinensis*	KC584201	KC584121	KC584663	KC584405	–	–	–
	A. nepalensis								
	CBS 118700; EGS45.073[T]	Nepal, *Brassica* sp.	KC584207	KC584126	KC584672	KC584414	–	–	–
Nimbya	*A. scirpicola* (= *N. scirpicola*)								
	CBS 481.90; EGS19.042[R]	UK, *Scirpus* sp.	KC584237	KC584163	KC584728	KC584469	–	–	–
Panax	*A. avenicola*								
	CBS 121459; EGS50.185[T]	Norway, *Avena* sp.	KC584183	KC584100	KC584639	KC584380	–	–	–
	A. dendropanacis								
	CNU 085031[T]	Korea, *Dendropanax morbifer*	HQ203210	KF516506	KP877992	KP877985	KF516492	–	–
	CNU 085033	Korea, *Aralia elata*	–	KF516507	KP877993	KP877986	KF516493	–	–
	A. eryngii								
	CBS 121339; EGS41.005; BMP0336[R]	unknown, *Eryngium* sp.	JQ693661	AY562416	KC584656	KC584397	AY563313	–	JQ671670
	A. panax								
	MUCC 1692; AC18; PFAlt1-1	**Japan, *Polyscias fruticosa***	–	*	*	*	*	–	*
	AC19; PGAlt1	**Japan, *Polyscias guilfoylei***	**LC440598**	*	*	*	*	–	*
	MAFF 243161; MUCC 1625; AC83	**Japan, *Polyscias fruticosa***	**LC440599**	AB862972	AB862984	AB862978	*	–	*
	MAFF 243162; MUCC 1626; AC84	**Japan, *Polyscias fruticosa***	**LC440600**	*	*	*	*	–	*
	CBS 482.81; EGS29.180[R]	USA, *Aralia racemosa*	KC584209	KC584128	KC584675	KC584417	JQ646382	–	JQ671672
	CBS 116532; EGS46.157[R] [as *A. araliae* in Deng *et al.* (2015)]	New Zealand, *Meryta sinclairii*	JF417549	JF417630	JX213321	JF417657	JX213285	–	–
	CBS 116535; EGS48.124[R]	USA, *Panax quinquefolius*	JF417562	JF417643	JX213334	JF417670	JX213298	–	–
	CNU 085019	Korea, *Panax ginseng*	–	KF516502	KP877988	KP877981	KF516488	–	–

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	CNU 101004 [as *A. araliae* in Deng *et al.* (2015)]	Korea, *Aralia continentalis*	–	KF516501	KP877987	KP877980	KF516487	–	–
	A. photistica								
	CBS 212.86; EGS35.172; BMP0041[T]	UK, *Digitalis purpurea*	KC584212	KC584131	KC584678	KC584420	AY563282	–	JQ671632
Phragmosporae	*A. phragmospora* (= *E. phragmospora*)								
	CBS 274.70; EGS27.098[T]	Netherlands, soil	JN383493	JN383474	KC584721	KC584462	JN383509	–	JQ671623
Porri	*Alternaria allii*								
	CBS 107.28; EGS48.084[T]	Puerto Rico, *Allium cepa*	KJ718100	KJ717954	KJ718449	KJ718274	KJ718620	–	–
	CBS 116701; EGS33.134[R]	USA, *Allium cepa* var. *viviparum*	KJ718103	KJ717957	KJ718452	KJ718277	KJ718623	–	–
	A. crassa								
	MAFF 243056; AC4	**Japan, *Datura stramonium***	AB678215	AB744032	*	*	AB744028	–	AB744035
	MUCC 2502; AC131	**Japan, *Datura fastuosa***	**LC440601**	*	*	*	*	–	*
	MUCC 2503; AC132	**Japan, *Datura inoxia***	–	*	*	*	*	–	*
	CBS 110.38[ET]	Cyprus, *Datura stramonium*	KJ718147	KJ717997	KJ718495	KJ718320	KJ718665	–	–
	CBS 109160; EGS45.075; BMP0180 (= *A. capsici*[T])	Australia, *Capsicum annuum*	KJ718148	AY562408	KJ718496	KJ718321	AY563298	–	JQ671747
	A. cucumerina								
	AC105	**Japan, *Cucurbita maxima***	**LC440602**	*	*	*	*	–	–
	AC106	**Japan, *Cucurbita maxima***	**LC440603**	*	*	*	*	*	–
	CBS 117225; EGS41.127[R]	USA, *Cucumis melo*	KJ718154	KJ718001	KJ718502	KJ718327	KJ718669	–	–
	CBS 116114; EGS35.123 (= *A. loofahae*[T])	USA, *Luffa acutangula*	KJ718153	KJ718000	KJ718501	KJ718326	KJ718668	–	–
	A. dauci								
	MUCC 1684; AC8	**Japan, *Daucus carota***	**LC440604**	*	*	*	*	–	*
	AC9	**Japan, *Daucus carota***	**LC440605**	*	*	*	*	–	*
	CBS 111.38[NT]	Italy, *Daucus carota*	KJ718158	KJ718005	KJ718506	KJ718331	KJ718673	–	–

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	A. macrospora								
	CBS 117228; EGS50.190[T]	USA, *Gossypium barbadense*	KC584204	KC584124	KC584668	KC584410	KJ718702	–	–
	A. porri								
	AC2	**Japan, *Viola × wittrockiana***	**LC440606**	*	*	*	*	–	*
	AC6	**Japan, *Calibrachoa* sp.**	**LC440607**	*	*	*	*	–	*
	MUCC 1688; AC14	**Japan, *Allium fistulosum***	**LC440608**	*	*	–	–	*	*
	AC16	**Japan, *Allium fistulosum***	**LC440609**	*	*	*	*	*	*
	AC17	**Japan, *Allium fistulosum***	–	*	*	*	*	–	*
	MUCC 1698; AC30	**Japan, *Allium fistulosum***	**LC440610**	*	*	*	*	*	*
	AC32	**Japan, *Allium fistulosum***	**LC440611**	*	*	*	*	*	*
	MUCC 1702; AC35	**Japan, *Eustoma exaltatum***	**LC440612**	*	*	*	*	*	*
	AC68	**Japan, *Allium fistulosum***	**LC440613**	*	*	*	*	*	*
	CBS 116699; EGS48.152[ET]	USA, *Allium cepa*	KJ718218	KJ718053	KJ718564	KJ718391	KJ718727	–	KJ718727
	CBS 116698; EGS48.147[R]	USA, *Allium cepa*	DQ323700	KC584132	KC584679	KC584421	KJ718726	–	–
	A. pseudorostrata								
	CBS 119411; EGS42.060; BMP0174[T]	USA, *Euphorbia pulcherrima*	JN383483	AY562406	KC584680	KC584422	AY563295	–	JQ671737
	A. solani								
	CBS 109157; EGS44.098[R]	USA, *Solanum tuberosum*	KJ718238	GQ180080	–	KJ718413	KJ718746	–	–
	A. tagetica								
	CBS 479.81; EGS33.081[R]	UK, *Tagetes erecta*	KC584221	KC584143	KC584692	KC584434	KJ718761	–	–
	A. zinniae								
	MUCC 1704; AC44	**Japan, *Zinnia hybrida***	**LC440614**	*	*	*	*	–	*
	AC107	**Japan, *Zinnia hybrida***	**LC440615**	*	*	*	*	–	–

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	AC108	**Japan, *Zinnia elegans***	**LC440616**	*	*	*	*	–	–
	AC109	**Japan, *Zinnia elegans***	**LC440617**	*	*	*	*	–	–
	CBS 117223; EGS44.035[R]	New Zealand, *Zinnia elegans*	KJ718270	KJ718096	KJ718616	KJ718445	KJ718777	–	–
Pseudoalternaria	*A. rosae*								
	CBS 121341; EGS41.130[T]	New Zealand, *Rosa rubiginosa*	JQ693639	JQ646279	JQ672414	–	JQ646370	–	JQ671628
Pseudoulocladium	*A. chartarum* (= *Ulocladium chartarum*)								
	MAFF 246888; AC85	**Japan, *Capsicum annuum***	**LC440618**	*	*	*	*	–	*
	CBS 200.67; ATCC 18044; BMP0359[ET]	Canada, *Populus* sp.	AF229488	KC584172	KC584741	KC584481	AY563319	–	JQ671654
	A. aspera (= *Ul. arborescens*)								
	CBS 115269; EGS44.109[T]	Japan, *Pistacia vera*	KC584242	KC584166	KC584734	KC584474	KF533899	–	–
	A. concatenata (= *Ul. capsici*)								
	CBS 120006; HSAUPIII$_0$0035[T]	China, *Capsicum annuum*	KC584246	AY762950	KC584740	KC584480	–	–	–
	A. septospora (= *Ul. septosporum*)								
	CBS 109.38	Italy, wood pulp	FJ266489	FJ266500	KC584747	KC584487	–	–	–
Radicina	*A. petroselini*								
	MAFF 243057; AC42	**Japan, *Petroselinum crispum***	AB678216	–	*	*	AB744030	–	AB744037
	CBS 112.41; EGS06.196[T]	unknown, *Petroselinum sativum*	KC584211	KC584130	KC584677	KC584419	–	–	–
	CBS 109383; EGS09.159; BMP0144[R]	USA, *Petroselinum crispum*	AF229454	AY278799	JQ672455	JQ646474	AY563288	–	JQ671677
	A. radicina								
	CBS 245.67; EGS03.145; ATCC 6503[NT]	USA, *Daucus carota*	KC584213	KC584133	KC584681	KC584423	FN689405	–	–
	A. selini								
	CBS 109382; EGS25.198[T]	Saudi Arabia, *Petroselinum crispum*	AF229455	AY278800	KC584684	KC584426	FJ266504	–	JQ671676

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	A. smyrnii								
	CBS 109380; EGS37.093; BMP0147[R]	UK, *Smyrnium olusatrum*	AF229456	KC584138	KC584687	KC584429	AY563289	–	JQ671675
Soda	*A. kulundii*								
	CBS 137525; M313[T]	Russia, soil	KJ443262	KJ649618	KJ443219	KJ443176	–	–	–
Sonchi	*A. cinerariae*								
	MAFF 243059; MUCC 1701; AC3[ET]	**Japan, *Pericallis cruenta***	**AB906673**	AB906670	*	*	**AB906671**	–	**AB906672**
	MAFF 241266; MUCC 1613; AC57	**Japan, *Farfugium japonicum***	**LC440619**	AB862970	AB862982	AB862976	*	–	*
	MAFF 241267; MUCC 1614; AC58	**Japan, *Gynura bicolor***	**LC440620**	AB862971	AB862983	AB862977	*	–	*
	MUCC 2504; AC138	**Japan, *Jacobaea maritima***	**LC440621**	*	–	*	*	–	*
	CBS 116495; EGS49.102[R]	USA, *Ligularia* sp.	KC584190	KC584109	KC584648	KC584389	–	–	–
	A. sonchi								
	CBS 119675; EGS43.131[R]	Canada, *Sonchus asper*	KC584220	KC584142	KC584691	KC584433	–	–	–
Teretispora	*A. leucanthemi* (= *Teretispora leucanthemi*)								
	CBS 421.65; ATCC 16028; EGS10.059[T]	Netherlands, *Chrysanthemum maximum*	KC584240	KC584164	KC584732	KC584472	–	–	–
Ulocladioides	*A. atra* (= *Ul. atrum*)								
	AC86	**Japan, *Raphanus sativus***	**LC440622**	*	*	*	*	–	*
	AC87	**Japan, *Brassica oleracea* var. *capitata***	–	*	*	*	*	–	*
	AC88	**Japan, *Brassica rapa* var. *pekinensis***	**LC440623**	*	*	*	*	–	*
	MAFF 246889; AC90	**Japan, *Allium fistulosum***	**LC440624**	*	*	*	*	–	*
	CBS 195.67; ATCC 18040; BMP0355[ET]	USA, soil	AF229486	KC584167	KC584735	KC584475	AY563318	–	JQ671660
	A. cucurbitae (= *Ul. cucurbitae*)								
	CBS 483.81; EGS31.021; BMP0351[R]	New Zealand, *Cucumis sativus*	FJ266483	AY562418	KC584743	KC584483	AY563315	–	JQ671663

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	A. multiformis (= *Ul. multiforme*)								
	CBS 102060; EGS31.005[T]	Canada, soil	FJ266486	KC584174	KC584744	KC584484	FJ266512	–	JQ671664
	A. cantlous (= *Ul. cantlous*)								
	CBS 123007; HSAUP0209[T]	China, *Cucumis melo*	KC584245	KC584171	KC584739	KC584479	EU684146	–	–
	A. heterospora (= *Ul. solani*)								
	CBS 123376; HSAUP 0521[T]	China, *Solanum lycopersicum*	KC584248	KC584176	KC584748	KC584488	EU855805	–	–
Ulocladium	*A. alternariae* (= *Sinomyces alternariae*)								
	CBS 126989; EGS46.004	USA, *Daucus carota*	AY376642	AY376329	KC584730	KC584470	–	–	–
	A. botrytis (= *Ul. botrytis*)								
	MAFF 246887; AC52	**Japan, *Asparagus officinalis***	**LC440625**	*	*	*	*	–	*
	CBS 197.67; ATCC 18042[ET]	USA, air	KC584243	KC584168	KC584736	KC584476	–	–	–
	A. oudemansii (= *Ul. oudemansii*)								
	CBS 114.07; ATCC 18047; IMI 124940; MUCL 18563; QM 1744[T]	unknown	FJ266488	KC584175	KC584746	KC584486	FJ266514		
Undifilum	*A. bornmuelleri* (= *Undifilum bornmuelleri*)								
	DAOM 231361	Austria, *Securigera varia*	FJ357317	FJ357305	KC584751	KC584491	JN383516	–	JQ671610
Monotypic lineage	*A. argyranthemi*								
	CBS 116530; EGS44.033[T]	New Zealand, *Argyranthemum* sp.	KC584181	KC584098	KC584637	KC584378	–	–	–
	A. brassicae								
	AC29	**Japan, *Brassica rapa***	**LC440626**	AB862967	AB862979	AB862973	*	–	*
	MAFF 240791; AC47	**Japan, *Raphanus sativus***	**LC440627**	*	*	*	*	–	*
	MUCC 1615; AC62	**Japan, *Raphanus sativus***	**LC440628**	*	*	*	*	–	*
	CBS116528; EGS38.032[R]	USA, *Brassica oleracea*	KC584185	KC584102	KC584641	KC584382	–	–	–

Table 5.2. (Continued).

Alternaria section	Fungal name and isolate numbers[1,2]	Country, host plant	ITS	*gapdh*	*tef1*	*rpb2*	*Alt a 1*	*endoPG*	*act*
	A. dennisii (= *E. dennisii*)								
	CBS 476.90; EGS30.121^{T}	Isle of Man, *Senecio jacobaea*	JN383488	JN383469	KC584713	KC584454	JN383505	–	–
	A. helianthiinficiens								
	CBS 208.86; EGS36.184^{T}	USA, *Helianthus annuus*	JX101649	KC584120	EU130548	KC584403	–	–	–
	A. peucedani								
	CNU 111485^{T}	Korea, *Peucedanum japonicum*	KF728231	KF889361	–	–	KF889363	–	–
	A. solialidae								
	CBS 118387; EGS33.024^{T}	USA, soil	KC584218	KC584140	KC584689	KC584431	–	–	–
	A. thalictrigena								
	CBS 121712; CPC 13410^{T}	Germany, *Thalictrum* sp.	EU040211	KC584144	KC584694	KC584436	–	–	–
	A. thlaspis (= *E. thlaspis*)								
	EGS45.069^{T}	UK, *Thlaspis caerulescentis*	JN383495	JN383476	–	–	JN383510	–	JQ671607
	***Alternaria* sp.**								
	MAFF 246776; AC5^{T}	**Japan, *Bupleurum rotundifolium***	**LC440629**	*	*	*	*	–	*
	AC95	**Japan, *Bupleurum rotundifolium***	**LC440630**	*	*	*	*	–	*
out group	*Paradendryphiella salina* (= *E. annulata*)								
	CBS 302.84^{T}	North Sea, *Cancer pagurus*	JN383486	JN383467	KC584709	KC584450	–	–	JQ671591

[1] AC: Personal collection of JN; ATCC: American Type Culture Collection, Virginia, USA; BMP: Personal collection of Dr. B.M. Pryor, School of Plant Sciences, University of Arizona, Arizona, USA; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CNU: Culture Collection of Chungnam National University, Daejeon, Korea; CPC: Personal collection of Dr. P.W. Crous; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; EGS: Personal collection of Dr. E.G. Simmons; HSAUP: Department of Plant Pathology, Shandong Agricultural University, China; MAFF: Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Japan; MUCC (Japan): Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Japan; P: Personal collection of Dr. P. Inderbitzin, Department of Plant Pathology, Cornell University, New York, USA.

[2] Ex-type, -neotype, and -epitype strain indicated with T, NT, and ET; R: representative strain by Simmons (2007); Fungal names between parentheses refer to the former name or the name under the pathotype concept (Nishimura 1980).

[3] Japanese isolates examined and accession numbers newly generated in this study are indicated in boldface. Asterisks indicate that registrations of sequences are incomplete.

To evaluate the validity of ITS as the fungal barcoding gene for *Alternaria*, MP, ML, and BI analyses were conducted with the ITS dataset, which was composed of 74 sequences generated from our collected Japanese isolates and other sequences from GenBank (Table 5.2). MP analyses were performed in PAUP* v. 4.0b10, with the same procedure and settings. ML analyses were performed in RAxML v. 8.1.17 (Stamatakis 2014), using the GTR+GAMMA model as the nucleotide substitution model and 100 BS replicates. BI analyses were performed in BEAST v. 2.5.1, with the HKY+GAMMA model selected by Kakusan4. To estimate the PPs of tree topologies, MCMCMC were run for 20 million generations with trees sampled and saved every 1000 generations. After discarding the initial 10,000 trees as burn-in, PPs were determined from the remaining trees. Sequences of *P. salina* were used as the outgroup.

To analyze the detailed relationships between Japanese isolates within sect. *Alternaria*, MP, ML, and BI analyses were conducted using a combined dataset of *act*, *Alt a 1*, *endoPG*, *gapdh*, *rpb2*, and *tef1* sequences, which was composed of nine sequences generated from our collected Japanese isolates and other sequences from GenBank (Table 5.2). MP and ML analyses were performed in PAUP* v. 4.0b10 and RAxML v. 8.1.17, respectively, using the same procedure and settings used for ITS analyses. BI analyses were performed in BEAST v. 2.5.1 with the GTR+GAMMA model selected by Kakusan4. To estimate the PPs of tree topologies, MCMCMC were run for 10 million generations with trees sampled and saved every 1000 generations, which generated 9001 trees from which the initial 1000 trees were discarded as burn-in. After discarding, PPs were determined from the remaining trees. Sequences of the Japanese isolate of *A. nobilis* (AC1) were also used as the outgroup. The generated trees were printed with FigTree v. 1.4.2 (Institute of Evolutionary Biology, University of Edinburgh, http://tree.bio.ed.ac.uk/software/figtree).

Inoculation tests

To determine the experimental host range of the obtained isolates, conidia that were artificially produced on V8 medium as described above were washed with sterile distilled water containing 0.02 % polyoxyethylene (20) sorbitan monolaurate (Wako Pure Chemicals, Osaka),

and released conidia were used as inocula (Nishikawa & Nakashima 2013). Concentrations of each conidial suspension were adjusted using a hemocytometer, and then each inoculum was sprayed onto the mature leaves of potted plants (at least three replicates). Closely related plant species from the same family as the original host source of each *Alternaria* species were inoculated. Furthermore, the unrelated plant species recorded as hosts were also inoculated to find potential host species, and to define host range boundaries of *Alternaria* species. All the inoculated plants were maintained in an incubator under moist conditions at 20 °C.

Virulent phenotypes were evaluated 7 d post-inoculation (dpi) using the index described by Chaerani *et al.* (2007) (0: no visible leaf lesions; 1: up to 10 % of leaf area affected; 2: 11–25 % of leaf area affected; 3: 26–50 % of leaf area affected; 4: 51–75 % of leaf area affected; and 5: more than 75 % of leaf area affected or the leaf abscised), and the means for each inoculated plant species were calculated as disease severity with 95 % confidence intervals. Given the importance of epidemiology to the obtained results, we also focused on whether sporulation was present or absent on the host lesions, and whether it was pure from inocula or with those of some saprophytes, to evaluate the host ranges more accurately. Inoculated plants showing no symptoms within 7 dpi were observed continuously until 30 dpi.

RESULTS

Morphology

Based on their conidial morphology on PCA and V8 media, the Japanese *Alternaria* isolates examined in the present study were recognized as either one of 23 existing species, or one of three novel species. One of the novel taxa, MAFF 246768 ex *Gomphrena haageana*, produced very similar conidia to those of *A. gomphrenae* and other species in sect. *Alternantherae*; however, they differed in the length and width of the conidial bodies (Table 5.3). Among their various features, conidiophore width was a defining characteristic of each *Alternaria* section: those of sect. *Alternaria* (*A. alstroemeriae*, *A. alternata*, *A. gaisen*, and MAFF 246770),

Table 5.3. Morphological comparisons among the species of sect. *Alternantherae*.

Fungal species and isolates[1]	Original host plants	Conidial bodies				Beaks		Substrates	References
		Length × width (μm)	Average with 95 % CI	Transverse septa	Longitudinal septa	Length × width (μm)	Average with 95 % CI		
Examined species									
Alternaria celosiicola	*Celosia*								
MAFF 243058		36–161 × 8–26	107±7 × 20±1	2–16	0–4	55–670 × 2–3	332±44 × 2±0.1	PCA	Nishikawa & Nakashima (2013)
		42–180 × 10–26	116±7 × 18±1	2–17	0–5	49–575 × 2–4	195±38 × 2±0.1	V8	Nishikawa & Nakashima (2013)
		68–173 × 13–26	119±5 × 20±1	8–15	0(–2)	120–285 × 2–3	197±13 × 2±0.1	Lesion	Nishikawa & Nakashima (2013)
EGS42.013^{T}		50–190 × 7–17	—	11–14	1–3	250–470 × 2–4	—	PCA	Simmons (1995)
A. gomphrenae	*Gomphrena*								
MAFF 246769^{ET}		46–103 × 11–21	82±4 × 16±1	4–10	0(–1)	18–188 × 2–4	87±10 × 3±0.1	PCA	This study
		35–77 × 10–17	58±2 × 14±0.4	3–9	0–1	13–216 × 2–4	97±14 × 3±0.1	V8	This study
		30–106 × 8–23	67±5 × 12±1	0–13	0	11–163 × 2–5	72±9 × 2±0.2	Lesion	This study
—		46–94 × 10–16	74±3 × 14±0.4	4–10	0	—	—	Lesion	This study, lectotype
—		100 × 15	—	5–14	—	up to 150	—	Lesion	Togashi (1926), Simmons (1989)
EGS40.146		80–100 × 18–20	—	—	—	60–80 × 2	—	PCA	Simmons (1995b)
—		48–105 × 9–18	80 × 14	—	rare	33–111 × 2–3	69 × 3	Lesion	Yoshii (1933)
Alternaria sp.	*Gomphrena*								
MAFF 246768		60–111 × 15–25	87±3 × 20±1	2–9	0–3	14–208 × 2–5	129±11 × 3±0.1	PCA	This study
		48–98 × 17–33	76±3 × 25±1	3–7	0–4	25–316 × 3–5	150±18 × 3±0.2	V8	This study
		25–99 × 8–26	56±5 × 19±1	1–9	0–2	14–87 × 3–4	55±4 × 3±0.1	Lesion	This study
Comparative species									
A. alternantherae	*Alternanthera*								
EGS39.124^{NT}		50–115 × 8–20	—	6–10	0–2	350–470 × 2–4	—	PCA	Simmons (1995b)
A. crassoides	*Froelichia*								
—		45–90 × 8–20	—	7–9	—	40–60 × 2	—	Lesion	Simmons (1995b), lectotype
A. perpunctulata	*Alternanthera*								
CBS 115267^{T}		80–100 × 10–14	—	10–15	1–2	100–210	—	PCA	Simmons (2004)

[1] CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; EGS: Personal collection of Dr. E.G. Simmons; MAFF: Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Japan. Ex-type, -neotype, and -epitype strain indicated with T, NT, and ET.

Brassicicola, *Crivellia*, *Japonica*, *Pseudoulocladium*, *Ulocladioides*, *Ulocladium*, and MAFF 246776 for the most part did not exceed an average of 5 µm (narrow conidiophores); those of sect. *Alternantherae* (*A. celosiicola*, *A. gomphrenae*, and MAFF 246768), *Eureca*, *Gypsophilae*, *Panax*, *Porri* (*A. crassa*, *A. cucumerina*, *A. dauci*, *A. porri*, *A. zinniae*), *Sonchi*, and *A. brassicae* usually reached 6–7 µm (thick conidiophores); those of sect. *Radicina* were of an intermediate width, ranging around 5–6 µm; and *A. iridicola* produced mostly narrowed, but often thickened, conidiophores. In addition, species in sect. *Porri* were characterized by the morphology of their beaks, especially in color. Those of *A. porri* and *A. dauci* were typical of hyaline filamentous, whereas those of *A. cucumerina* and *A. zinniae* were typical filamentous but always colored. Moreover, *A. crassa* grown on V8 medium commonly also formed colored beaks as cylindrical secondary conidiophores, but not filamentous true beaks. A detailed morphology of each Japanese species examined in the present study follows in the taxonomy section.

Growth rate on potato-dextrose agar

Colony diameters of the examined species ranged from 24–87 mm after 7 d incubation at 25 °C, and the mean with 95 % confidence intervals was 61.6 ± 6.3 mm (Fig. 5.1). Based on the mean colony diameters, the examined species were classified into groups; fast-growing: *A. alstroemeriae*, *A. alternata*, *A. celosiicola*, *A. crassa*, *A. cucumerina*, *A. gaisen*, *A. petroselini*, and *A. porri*; moderate-growing: *A. atra*, *A. botrytis*, *A. brassicicola*, *A. chartarum*, *A. cinerariae*, *A. cumini*, *A. iridicola*, *A. japonica*, *A. zinniae*, MAFF 246768 ex *Gomphrena*, and MAFF 246770 ex *Petunia*; slow to moderate-growing: *A. dauci*, *A. gomphrenae*, and *A. panax*; slow-growing: *A. brassicae*, *A. nobilis*, *A. penicillata*, and MAFF 246776 ex *Bupleurum*.

Molecular phylogeny

Combined gapdh, rpb2, and tef1 phylogeny

The combined alignment of the *gapdh, rpb2, and tef1* datasets contained 189 sequences with a total of 1567 characters. The topologies of the resulting trees from MP, ML, and BI analyses

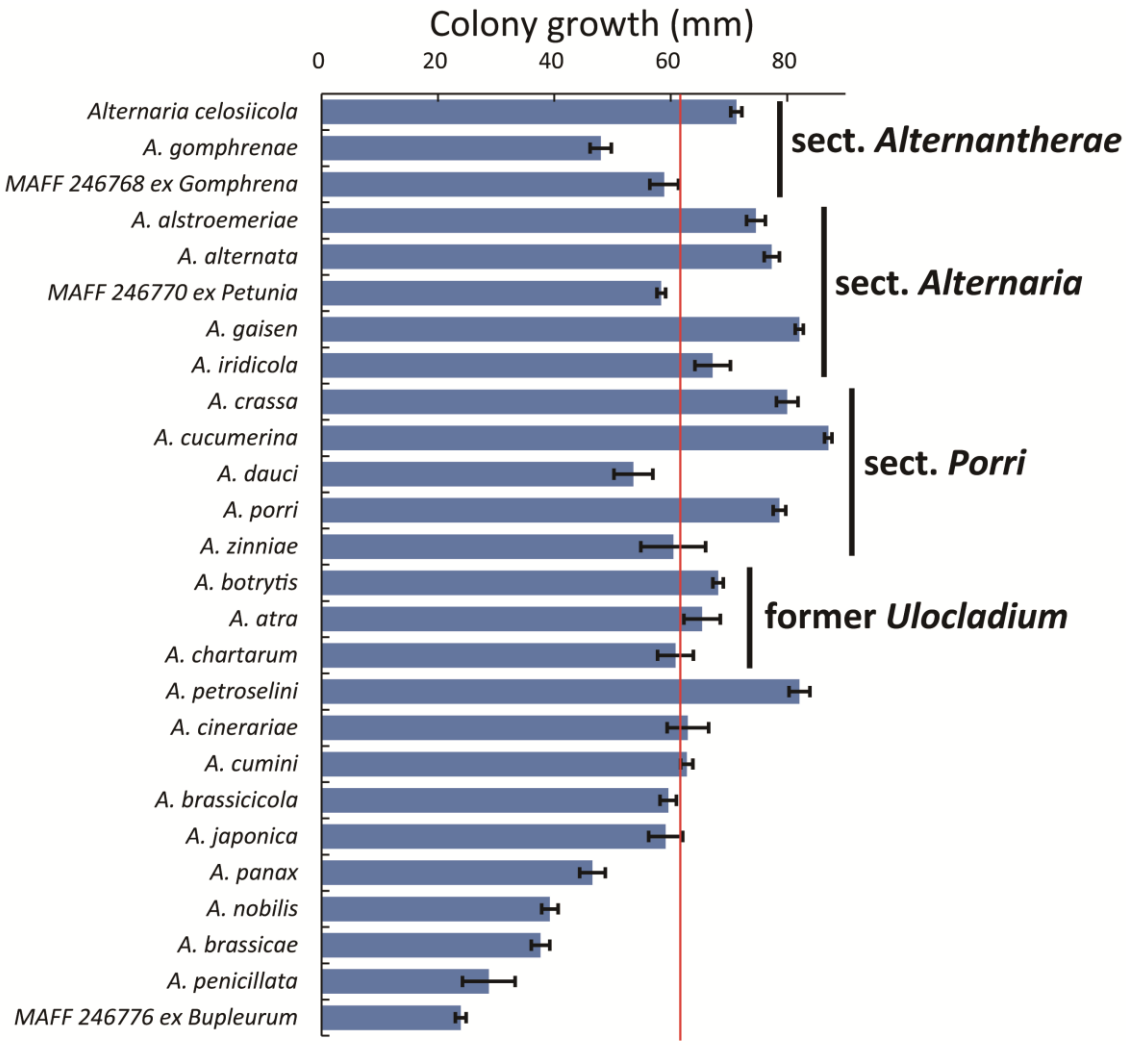

Fig. 5.1. Mean colony diameters (mm) on PDA. The mean for the entire examined species is indicated as a red line at 61.6 mm. Bars indicate the 95 % confidence intervals.

were congruent, and Fig. 5.2 shows the ML tree with BS values (MP and ML) and Bayesian PP. The Japanese *Alternaria* isolates examined were divided into 14 sections and two monotypic lineages as strongly supported clades. Five Japanese species were clustered together into sect. *Alternaria* and *Porri* each, and three species into sect. *Alternantherae*. Among the three novel species identified by their distinct morphological characteristics, MAFF 246768 ex *G. haageana* was clearly distinguishable from *A. gomphrenae* and *A. celosiicola* in sect. *Alternantherae*, and

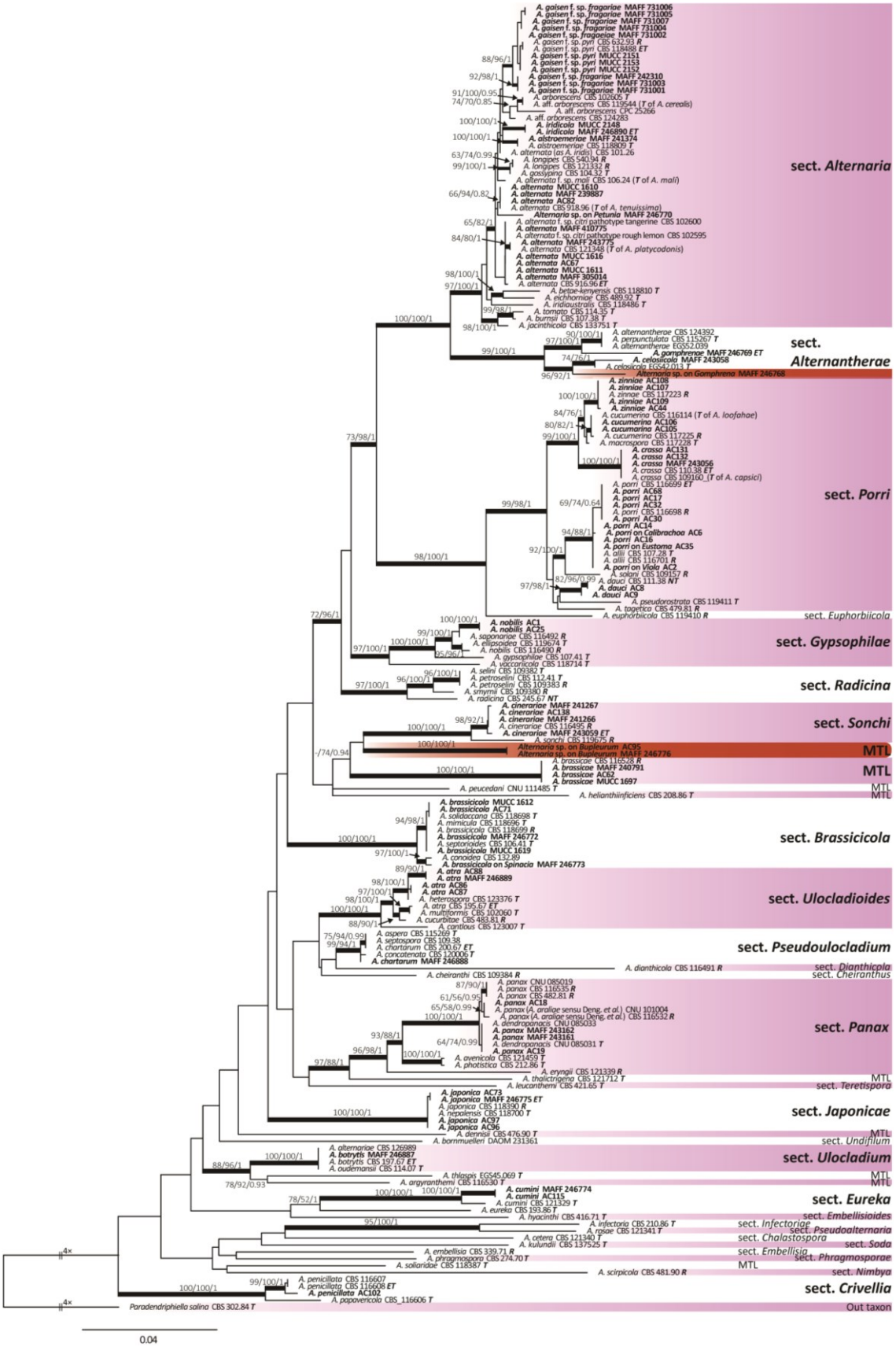
A. gaisen f. sp. fragariae MAFF 731006
A. gaisen f. sp. fragariae MAFF 731005
A. gaisen f. sp. fragariae MAFF 731007
A. gaisen f. sp. fragariae MAFF 731004
A. gaisen f. sp. fragariae MAFF 731002
A. gaisen f. sp. pyri CBS 632.93 R
A. gaisen f. sp. pyri CBS 118488 ET
A. gaisen f. sp. pyri MUCC 2151
A. gaisen f. sp. pyri MUCC 2153
A. gaisen f. sp. pyri MUCC 2152
A. gaisen f. sp. fragariae MAFF 242310
A. gaisen f. sp. fragariae MAFF 731003
A. gaisen f. sp. fragariae MAFF 731001
A. arborescens CBS 102605 T
A. aff. arborescens CBS 119544 (T of A. cerealis)
A. aff. arborescens CPC 25266
A. aff. arborescens CBS 124283
A. iridicola MUCC 2148
A. iridicola MAFF 246890 ET
A. alstroemeriae MAFF 241374
A. alstroemeriae CBS 118809 T
A. alternata (as A. iridis) CBS 101.26
A. longipes CBS 540.94 R
A. longipes CBS 121332 R
A. gossypina CBS 104.32 T
A. alternata f. sp. mali CBS 106.24 (T of A. mali)
A. alternata MUCC 1610
A. alternata MAFF 239887
A. alternata AC82
A. alternata CBS 918.96 (T of A. tenuissima)
Alternaria sp. on Petunia MAFF 246770
A. alternata f. sp. citri pathotype tangerine CBS 102600
A. alternata MAFF 410775
A. alternata f. sp. citri pathotype rough lemon CBS 102595
A. alternata MAFF 243775
A. alternata CBS 121348 (T of A. platycodonis)
A. alternata MUCC 1616
A. alternata AC67
A. alternata MUCC 1611
A. alternata MAFF 305014
A. alternata CBS 916.96 ET
A. betae-kenyensis CBS 118810 T
A. eichhorniae CBS 489.92 T
A. iridiaustralis CBS 118486 T
A. tomato CBS 114.35 T
A. burnsii CBS 107.38 T
A. jacinthicola CBS 133751 T
sect. Alternaria
A. alternantherae CBS 124392
A. perpunctulata CBS 115267 T
A. alternantherae EGS52039
A. gomphrenae MAFF 246769 ET
A. celosicola MAFF 243058
A. celosicola EGS42013 T
sect. Alternantherae
Alternaria sp. on Gomphrena MAFF 246768
A. zinniae AC108
A. zinniae AC107
A. zinniae CBS 117223 R
A. zinniae AC109
A. zinniae AC44
A. cucumerina CBS 116114 (T of A. loofahae)
A. cucumerina AC106
A. cucumarina AC105
A. cucumerina CBS 117225 R
A. macrospora CBS 117228 T
A. crassa AC131
A. crassa AC132
A. crassa MAFF 243056
A. crassa CBS 110.38 ET
A. crassa CBS 109160 (T of A. capsici)
A. porri CBS 116699 ET
A. porri AC68
A. porri AC17
A. porri AC32
A. porri CBS 116698 R
A. porri AC30
A. porri AC14
A. porri on Calibrachoa AC6
A. porri AC16
A. porri on Eustoma AC35
A. allii CBS 107.28 T
A. allii CBS 116701 R
A. porri on Viola AC2
A. solani CBS 109157 R
A. dauci CBS 111.38 NT
A. dauci AC8
A. dauci AC9
A. pseudorostrata CBS 119411 T
A. tageticа CBS 479.81 R
A. euphorbiicola CBS 119410 R
sect. Porri
sect. Euphorbiicola
A. nobilis AC1
A. nobilis AC25
A. saponariae CBS 116492 R
A. ellipsoidea CBS 119674 T
A. nobilis CBS 116490 R
A. gypsophilae CBS 107.41 T
A. vaccariicola CBS 118714 T
sect. Gypsophilae
A. selini CBS 109382 T
A. petroselini CBS 112.41 T
A. petroselini CBS 109383 R
A. smyrnii CBS 109380 R
A. radicina CBS 245.67 NT
sect. Radicina
A. cinerariae MAFF 241267
A. cinerariae AC138
A. cinerariae MAFF 241266
A. cinerariae CBS 116495 R
A. cinerariae MAFF 243059 ET
A. sonchi CBS 119675 R
sect. Sonchi
Alternaria sp. on Bupleurum AC95
Alternaria sp. on Bupleurum MAFF 246776
MTL
A. brassicae CBS 116528 R
A. brassicae MAFF 240791
A. brassicae AC62
A. brassicae MUCC 1697
MTL
A. peucedani CNU 111485 T
A. helianthiinficiens CBS 208.86 T
MTL
A. brassicicola MUCC 1612
A. brassicicola AC71
A. solidaccana CBS 118698 T
A. mimicula CBS 118696 T
A. brassicicola CBS 118699 R
A. brassicicola MAFF 246772
A. septoroides CBS 106.41 T
A. brassicicola MUCC 1619
A. conoidea CBS 132.89
A. brassicicola on Spinacia MAFF 246773
sect. Brassicicola
A. atra AC88
A. atra MAFF 246889
A. atra AC86
A. atra AC87
A. heterospora CBS 123376 T
A. atra CBS 195.67 ET
A. multiformis CBS 102060 T
A. cucurbitae CBS 483.81 R
A. cantlous CBS 123007 T
A. aspera CBS 115269 T
A. septospora CBS 109.38
A. chartarum CBS 200.67 ET
A. concatenata CBS 120006 T
A. chartarum MAFF 246888
sect. Ulocladioides
sect. Pseudoulocladium
A. dianthicola CBS 116491 R
sect. Dianthicola
A. cheiranthi CBS 109384 R
sect. Cheiranthus
A. panax CNU 085019
A. panax CBS 116535 R
A. panax CBS 482.81 R
A. panax AC18
A. panax (A. araliae sensu Deng, et al.) CNU 101004
A. panax (A. araliae sensu Deng, et al.) CBS 116532 R
A. dendropanacis CNU 085033
A. panax MAFF 243162
A. panax MAFF 243161
A. dendropanacis CNU 085031 T
A. panax AC19
A. avenicola CBS 121459 T
A. photistica CBS 212.86 T
sect. Panax
A. eryngii CBS 121339 R
A. thalictrigena CBS 121712 T
A. leucanthemi CBS 421.65 T
MTL
sect. Teretispora
A. japonica AC73
A. japonica MAFF 246775 ET
A. japonica CBS 118390 R
A. nepalensis CBS 118700 T
A. japonica AC97
A. japonica AC96
sect. Japonicae
A. dennisii CBS 476.90 T
MTL
A. bornmuelleri DAOM 231361
sect. Undifilum
A. alternariae CBS 126989
A. botrytis MAFF 246887
A. botrytis CBS 197.67 ET
A. oudemansii CBS 114.07 T
sect. Ulocladium
A. thlaspis EGS45.069 T
MTL
A. argyranthemi CBS 116530 T
MTL
A. cumini MAFF 246774
A. cumini AC115
A. cumini CBS 121329 T
A. eureka CBS 193.86 T
sect. Eureka
A. hyacinthi CBS 416.71 T
sect. Embellisioides
A. infectoria CBS 210.86 T
A. rosae CBS 121341 T
sect. Infectoriae
sect. Pseudoalternaria
A. cetera CBS 121340 T
A. kulundii CBS 137525 T
sect. Chalastospora
sect. Soda
A. embellisia CBS 339.71 R
A. phragmospora CBS 274.70 T
sect. Embellisia
sect. Phragmosporae
A. soliaridae CBS 118387 T
MTL
A. scirpicola CBS 481.90 R
sect. Nimbya
A. penicillata CBS 116607
A. penicillata CBS 116608 ET
A. penicillata AC102
A. papavericola CBS_116606 T
sect. Crivellia
Paradendriphiella salina CBS 302.84 T
Out taxon
0.04

Fig. 5.2. Maximum likelihood (ML) tree based on the combined dataset of *gapdh*, *rpb2*, and *tef1* sequences from Japanese *Alternaria* isolates. The tree was rooted to *Paradendryphiella salina* (CBS 302.84). Maximum parsimony (MP) and ML bootstrap values and Bayesian posterior probabilities (PP) are given near branches (MP/ML/PP). Thickened nodes indicate significant support by MP/ML/PP (> 70/70/0.95). The scale bar indicates the number of nucleotide substitutions per site. Japanese isolates examined are indicated in bold, and the statuses of reference isolates are indicated in bold and italic. *T*: ex-type, *NT*: ex-neotype, *ET*: ex-epitype, *R*: representative strain assigned by Simmons (2007). Names of sections and monotypic lineages (MTL) for each taxon are given in the right column, and the Japanese isolates examined in the study are also indicated in bold. Resolved novel taxa were indicated as red shadings.

two isolates (MAFF 246776 and AC95) ex *Bupleurum* were also well-resolved as a new monotypic sister lineages to sect. *Sonchi*. However, MAFF 246770 ex *Petunia* had a unique sequence with strong BS support in ML but with weak in MP and BI. The remaining other morphologically distinguishable species were assigned to each valid clade, whereas Japanese isolates of *A*. *atra*, *A*. *botrytis*, *A*. *brassicicola*, *A*. *chartarum*, *A*. *japonica*, *A*. *nobilis*, *A*. *panax*, and *A*. *porri* were indistinguishable from closely related taxa, including the ex-type and -epitype isolates in each section.

Species recognition based on ITS phylogeny

The ITS datasets containing 178 sequences were aligned to a total of 586 characters. The topologies of the resulting trees from MP, ML, and BI analyses were congruent, and Fig. 5.3 shows one of the MP trees (TL = 600, CI = 0.433, RI = 0.881, RC = 0.382, HI = 0.567) with BS values (MP and ML), and Bayesian PP. Almost all of the examined Japanese species, together with their closely related taxa in phylogenetic trees that were indistinguishable based on the combined *gapdh*, *rpb2*, and *tef1* sequence datasets, were each recognized as one species. Two isolates (MAFF 246776 and AC95) ex *Bupleurum* were well-resolved as a distinct new species.

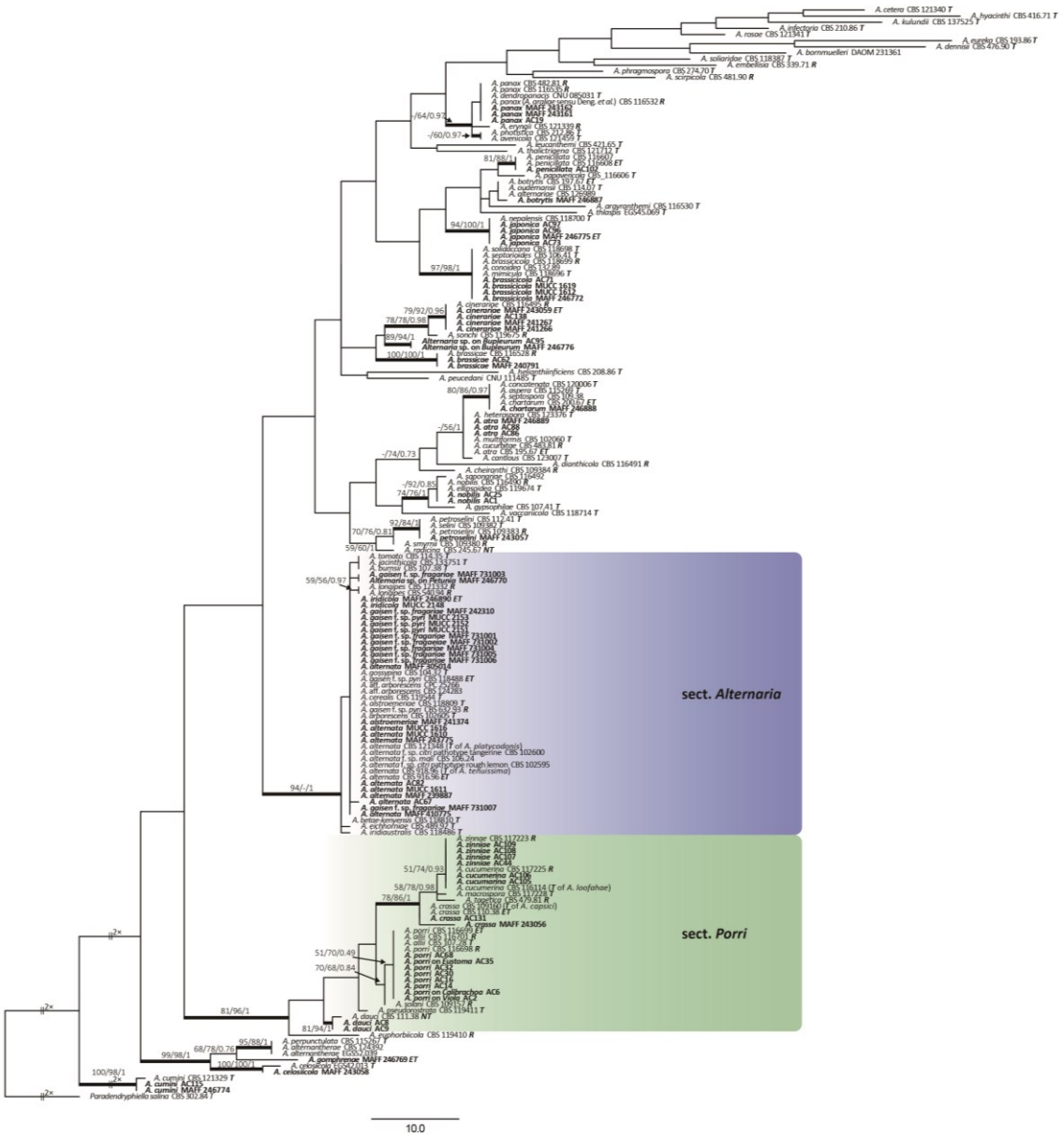

Fig. 5.3. Phylogenetic tree generated from maximum parsimony (MP) analysis based on the ITS sequences from Japanese *Alternaria* isolates. The tree was rooted to *Paradendryphiella salina* (CBS 302.84). MP and RAxML maximum likelihood (ML) bootstrap values and Bayesian posterior probabilities (PP) are given near branches (MP/ML/PP). Thickened nodes indicate significant support by MP/ML/PP (> 60/60/0.96). Tree length = 600, consistency index = 0.433, homoplasy index = 0.567, retention index = 0.881, and rescaled consistency index = 0.382. The scale bar indicates the number of nucleotide substitutions. Japanese *Alternaria* isolates examined are indicated in bold, and the statuses of reference isolates are indicated in bold and italic. *T*: ex-type, *NT*: ex-neotype, *ET*: ex-epitype, *R*: representative strain assigned by Simmons (2007).

However, the entire species in sect. *Alternaria*, and two large-spored species in sect. *Porri* having colored filamentous beaks (*A*. *cucumerina*, and *A*. *zinniae*), were not recognized as independent species.

Phylogeny of sect. **Alternaria**

The combined alignment of the *act*, *Alt a 1*, *endoPG*, *gapdh*, *rpb2*, and *tef1* datasets contained 18 sequences with a total 2473 characters. The topologies of the resulting trees from MP, ML, and BI analyses were congruent, and Fig. 5.4 shows one of the MP trees (TL = 383, CI = 0.859, RI = 0.783, RC = 0.673, HI = 0.141) with BS values (MP and ML) and Bayesian PP. MAFF 246770 ex *Petunia* was identified as a new sister lineage to the *A*. *arborescens* species complex in this section.

Experimental host range

Alternaria gomphrenae ***and a novel species infecting*** **Amaranthaceae**

Two *Alternaria* isolates (MAFF 246768 ex *G*. *haageana* and MAFF 246769 ex *G*. *globosa*) were applied to *Amaranthaceae* plants by spraying a conidial suspension concentrated at an average of 2.2×10^4 conidia/mL; both isolates had similar pathogenicity toward applied plants, but differed in their pathogenicity toward *Alternanthera sessilis* (Table 5.4). Distinct reddish spots appeared on *G. globosa* after 7 dpi with MAFF 246769, and then the inoculated leaves were defoliated, with poor sporulation on lesions even after 30 dpi (Fig. 5.5A, B). No distinct symptoms caused by MAFF 246769 were observed on the inoculated leaves of *Alternanthera* or the other examined plants—*Amaranthus tricolor*, two varieties of *Celosia argentea*, *Beta vulgaris*, and *Spinacia oleracea*—until 30 dpi (Fig. 5.5C). Distinct spots caused by MAFF 246768 similar to those of MAFF 246769 were also observed, but were more severe on *G. globosa* by 10 dpi (Fig. 5.5D). Indistinct spots frequently appeared on the leaves of *Alternanthera* inoculated with MAFF 246768 by 2 dpi, and then the leaves were severely defoliated with

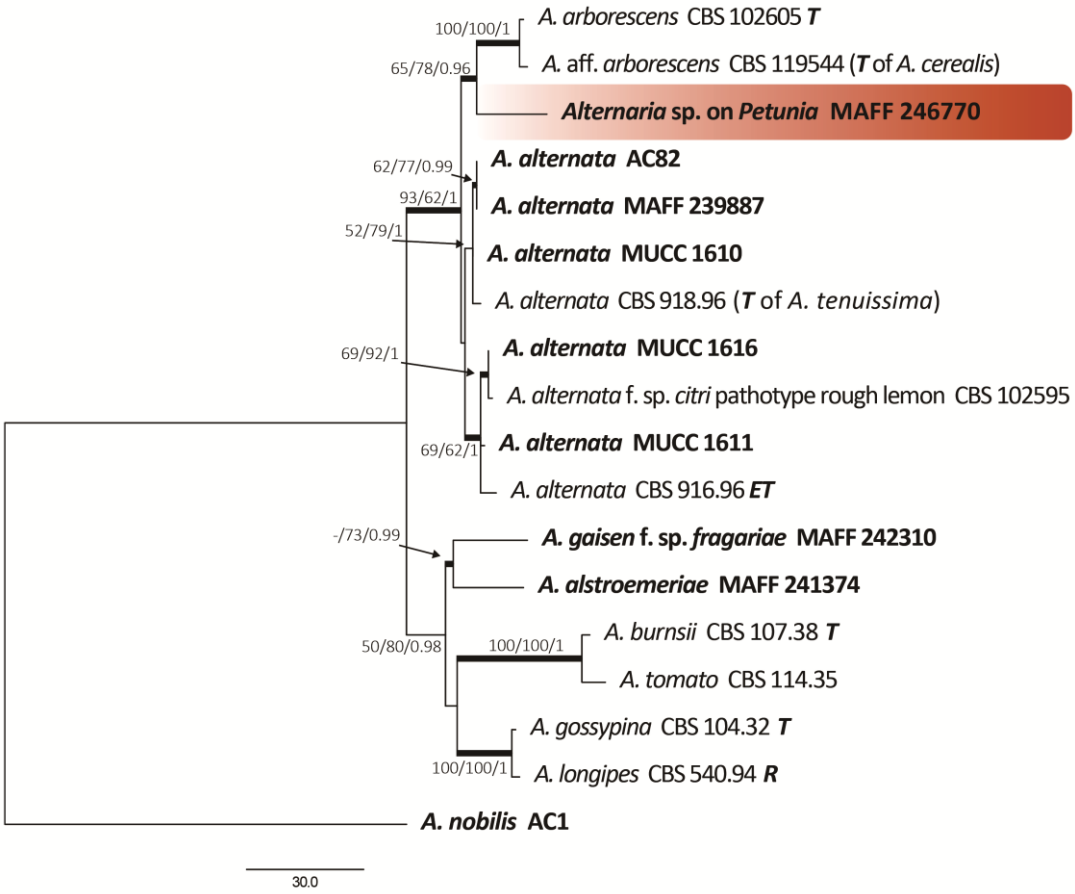

Fig. 5.4. Phylogenetic tree of sect. *Alternaria* generated from maximum parsimony (MP) analysis based on the combined dataset of *gapdh*, *tef1*, *rpb2*, *Alt a 1*, *endoPG*, and *act* sequences from 17 isolates. The tree was rooted to *Alternaria nobilis* (sect. *Gypsophilae*). MP and RAxML maximum likelihood (ML) bootstrap values and Bayesian posterior probabilities (PP) are given near branches (MP/ML/PP). Thickened nodes indicate significant support by MP/ML/PP (> 60/60/0.96). Tree length = 383, consistency index = 0.859, homoplasy index = 0.141, retention index = 0.783, and rescaled consistency index = 0.673. The scale bar indicates the number of nucleotide substitutions. Japanese isolates examined are indicated in bold, and statuses of reference isolates are indicated in bold and italic. *T*: ex-type, *ET*: ex-epitype, *R*: representative strain assigned by Simmons (2007).

Table 5.4. Experimental host ranges of *Alternaria* species infecting *Amaranthaceae*.

Inoculated plants	Disease severity[a] and pathogenicity[b] by inoculation with:				Notes[e]
	Novel species	*Alternaria gomphrenae*	*Alternaria celosiicola*[c]	*Alternaria alternantherae*[d]	
	MAFF 246768	MAFF 246769	MAFF 243058		
Amaranthaceae					
Amaranthoideae					
Amarantheae					No distinct symptoms were observed on the inoculated leaves with *Ago,* while *Ace* is pathogenic to plants in *Amaranthoideae*. *Aal* is non-pathogenic to *Amaranthus spinosus*, but to tribe *Celosieae*.
Amaranthus tricolor	0.1±0.2	0	4.4±0.6 ***	NT	
Celosieae					
Celosia argentea var. *cristata*	0.8±0.3 *	0	**4.6±0.5 *** **	S	
C. argentea var. *plumosa*	0.4±0.3	0	**4.7±0.3 *** **	S	
Betoideae					No distinct symptoms were observed on the inoculated leaves with *Ace* and *Ago*, but *Aal* was reported as pathogenic to both *Beta* and *Spinacia*.
Beta vulgaris	0	0.3±0.3	0.2±0.3	S	
Chenopodioideae					
Spinacia oleracea	0.1±0.2	0	0.1±0.2	S	
Gomphrenoideae					Distinct leaf spots and defoliations with rich sporulation were observed on *Gomphrena* inoculated with *Ago* isolates by 6 dpi. Leaf spots produced by *Ago* also observed on *Alternanthera* by 7 dpi but fewer with poor sporulation than on *Gomphrena*. *Ace* has distinct pathogenicity to *Alternanthera* and *Gomphrena*, but stronger than the former. *Aal* is partially pathogenic to *Alternanthera* species at least but not to *Gomphrena*.
Alternanthera sessilis	3.3±0.8 **	0.5±0.7 *	4.5±0.4 ***	**NT**	
Gomphrena globosa	**4.6±0.3 *** **	**2.0±0.5 *** **	3.0±0.7 **	I	

[a] Mean disease severity at 7 d post-inoculation (dpi) rated on a 0–5 scale (0: no visible lesions, 1: <10 % leaf area affected, 2: 11–25 % leaf area affected, 3: 26–50 % leaf area affected, 4: 51–75 % leaf area affected, and 5: >75 % leaf area affected or defoliation). 95 % confidence intervals are also indicated. NT: not tested. Results for the original plant source of each *Alternaria* isolate are indicated in bold.

[b] Pathogenicities were evaluated by the presence or absence of distinct lesions and sporulation on lesions, and are indicated with asterisks (***: strongly pathogenic, showing distinct lesions with rich sporulation; **: weakly pathogenic, showing indistinct or fewer distinct lesions with sporulation; *: weakly pathogenic to opportunistic, showing few, indistinct lesions with no to rare sporulation; blank: non-pathogenic, showing no distinct lesions nor sporulation).

[c] From results reported by Nishikawa & Nakashima (2013).
[d] From results reported by Pomella *et al.* (2007). S: susceptible, I: immune. They also determined *Alternanthera philoxeroides* and *Portulaca halimoides* (*Portulacaceae*) as susceptible hosts, and *Alternanthera ficoidea*, *Amaranthus spinosus*, and *Hebanthe eriantha* (= *Pfaffia paniculata*; *Gomphrenoideae*) as immune plants.
[e] Fungal names are abbreviated as follows: *Ace*: *Alternaria celosiicola*, *Ago*: *A*. *gomphrenae*, and *Aal*: *A*. *alternantherae*.

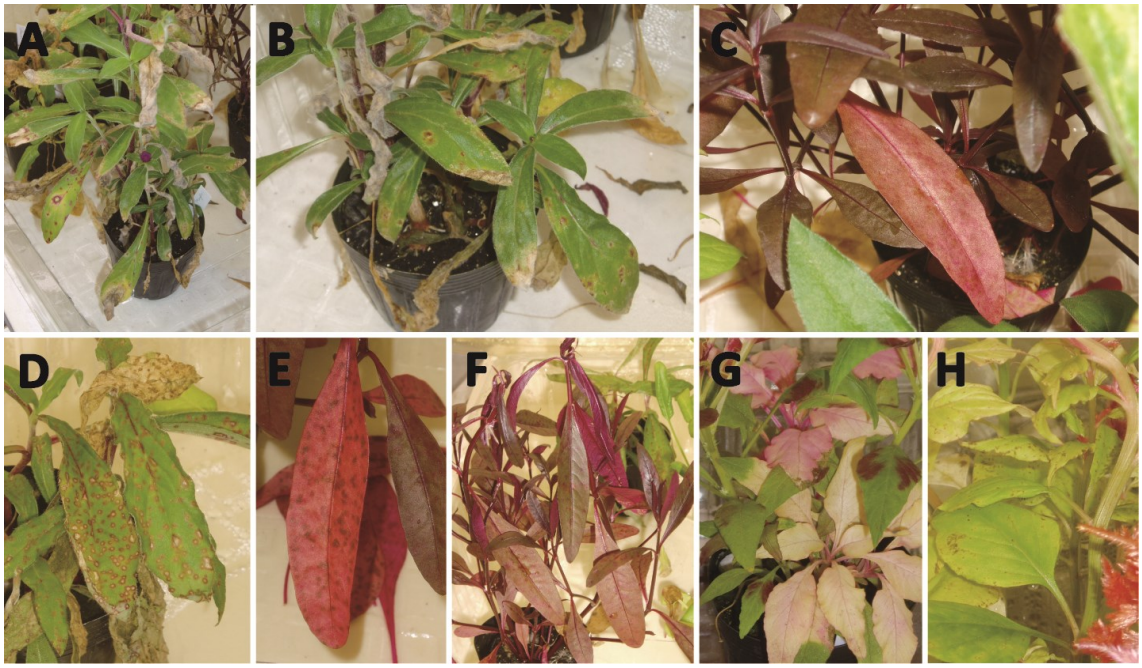

Fig. 5.5. Pathogenicity of two *Alternaria* species of sect. *Alternantherae*. **A–C.** *Alternaria gomphrenae* (MAFF 246769); **A, B.** On *Gomphrena* at 30 d post-inoculation (dpi). **C.** On *Alternanthera* at 8 dpi. **D–H.** *Alternaria* sp. (MAFF 246768); **D.** On *Gomphrena* at 10 dpi. **E, F.** On *Alternanthera* at 4–6 dpi. **G.** On *Amaranthus* at 11 dpi. **H.** *Celosia argentea* var. *cristata* at 4 dpi.

sporulation by 10 dpi (Fig. 5.5E, F). Almost no distinct symptoms were observed on the inoculated leaves of the other examined plants with MAFF 246768 until 30 dpi (Fig. 5.5G, H).

***Species infecting* Brassicaceae**

Three isolates of *A. brassicae*, MAFF 240791, AC29, and AC62, were applied by spraying conidial suspension concentrated at an average of 8.4×10^4 conidia/mL (Table 5.5). Distinct black lesions appeared on the inoculated leaves of all of the tribe *Brassiceae* plants, *Nasturtium officinale*, and *Iberis sempervirens* within 2 dpi, and then the inoculated leaves showed severe rot or defoliation with rich sporulation at 7–10 dpi. (Fig. 5.6A–H). Distinct black spots also appeared on *Eutrema japonicum* at 7 dpi, though these differed in disease severity between

applied isolates (Fig. 5.6I). Lesions on *Lobularia maritima* and *Matthiola incana* were usually indistinct; however, severe leaf blight or rot were observed with rich sporulation at 9–10 dpi (Fig. 5.6J, K). No distinct symptoms were observed on *Aubrieta* sp. and *Capsella bursa-pastoris* until 30 dpi as on the non-*Brassicaceae* plants, though poor sporulation was sometimes seen on lower aged leaves (Fig. 5.6L).

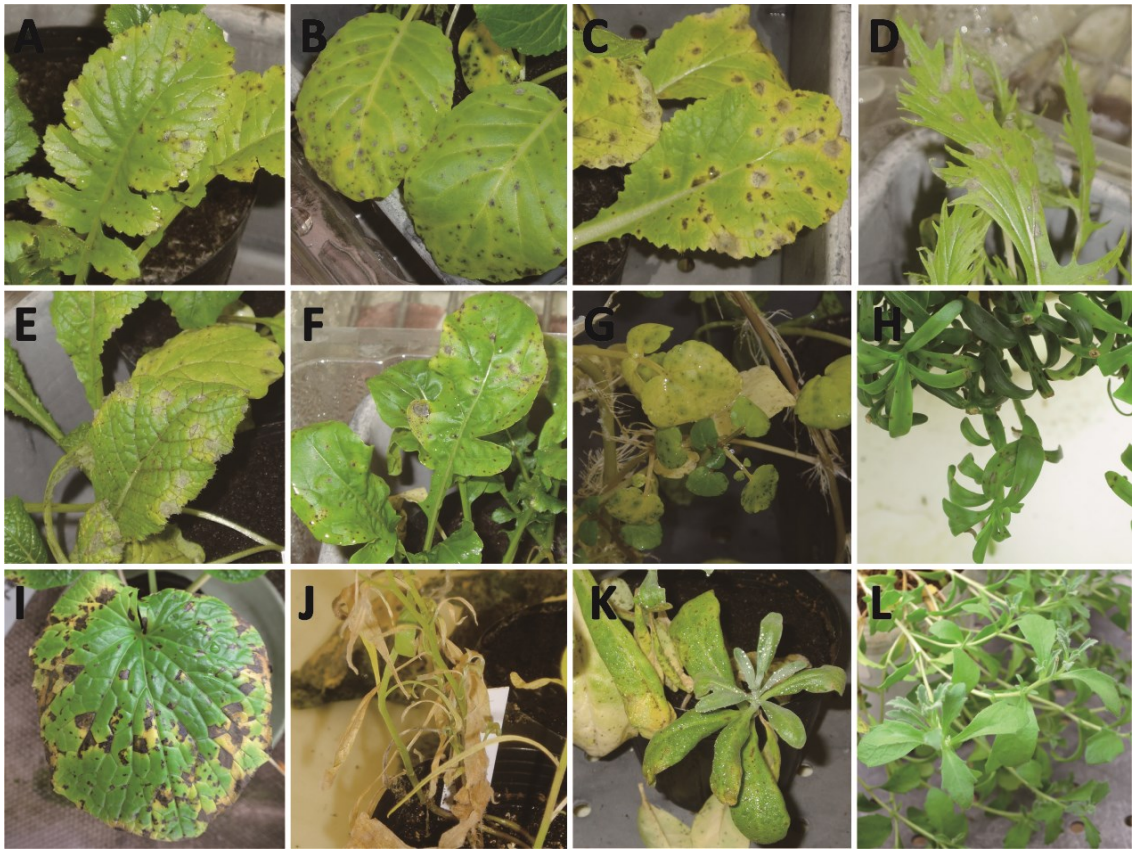

Fig. 5.6. Pathogenicity of *Alternaria brassicae* (MAFF 240791). **A.** On *Raphanus* at 5 d post-inoculation (dpi). **B.** On *Brassica oleracea* var. *capitata* at 5 dpi. **C.** On *B. rapa* subsp. *pekinensis* at 7 dpi. **D.** On *B. rapa* subsp. *nipposinica* at 5 dpi. **E.** On *B. juncea* at 5 dpi. **F.** On *Eruca* at 7 dpi. **G.** On *Nasturtium* at 2 dpi. **H.** On *Iberis* at 7 dpi. **I.** On *Eutrema* at 10 dpi. **J.** On *Lobularia* at 9 dpi. **K.** On *Matthiola* at 13 dpi. **L.** On *Aubrieta* at 7 dpi.

Table 5.5. Experimental host ranges of *Alternaria* species infecting *Brassicaceae*.

Inoculated plants	Disease severity[a] and pathogenicity[b] by inoculation with:								Notes[c]
	Alternaria brassicae			*Alternaria brassicicola*			*Alternaria japonica*		
	AC29	MAFF 240791	AC62	MAFF 246772	MAFF 246773	AC70	MAFF 246775	AC96	
Brassicaceae									
Alysseae									
Lobularia maritima	2.4±0.6 **	3.6±0.4 **	3.3±0.7 **	3.5±0.6 **	3.0±1.1 **	2.8±0.8 **	3.1±0.6 **	0.2±0.3 *	Small black spots and indistinct leaf blight were observed at 7–10 dpi, usually with rich sporulation.
Anchonieae									
Matthiola incana	3.1±0.4 **	3.7±0.5 **	2.6±0.5 **	4.9±0.2 ***	4.9±0.2 ***	4.8±0.3 ***	4.8±0.3 ***	3.8±0.4 ***	Black lesions appeared, then whole plants rotted with rich sporulation by 7 dpi, but relatively slight for *Abe*.
Arabideae									
Aubrieta sp.	0	0	0.2±0.2	0.3±0.2 *	1.4±0.7 *	0.3±0.2 *	1.9±0.5 *	0.1±0.2 *	Distinct symptoms were rarely observed. Isolate MAFF 246775 of *Aja* produced necrotic spots without sporulation at 7 dpi, and isolates of *Aba* often reproduced conidia on stem surfaces without distinct symptoms.
Brassiceae									
Brassica juncea	4.3±0.6 **	4.2±0.4 ***	3.3±0.5 ***	4.9±0.2 ***	4.4±0.5 ***	3.7±0.7 ***	2.8±0.6 ***	3.3±0.8 ***	Black spots were produced on leaves of all *Brassiceae* plants, then whole plants rotted with rich sporulation by 7 dpi. No significant differences were found between inoculated species.
B. oleracea var. *capitata*	4.1±0.4 ***	3.8±0.3 ***	4.0±0.5 ***	**4.9±0.1 *****	4.5±0.6 ***	5.0 ***	5.0 ***	**4.7±0.3 *****	
B .oleracea var. *sabellica*	NT	NT	NT	**4.6±0.2 *****	NT	NT	NT	**NT**	
B. rapa subsp. *chinensis*	**NT**	4.1±0.5 ***	NT	4.6±0.3 ***	4.1±0.5 ***	4.1±0.6 ***	4.1±0.7 ***	3.3±0.7 ***	
B. rapa subsp. *nipposinica*	**NT**	3.4±0.4 ***	NT	4.7±0.4 ***	5.0 ***	5.0 ***	3.7±0.5 ***	4.5±0.4 ***	
B. rapa subsp. *pekinensis*	**4.1±0.2 *****	4.0±0.3 ***	4.1±0.2 ***	4.3±0.4 ***	4.8±0.3 ***	4.4±0.6 ***	2.7±0.7 ***	3.6±0.6 ***	
B. rapa subsp. *rapa*	**3.4±0.3 *****	4.0±0.7 ***	3.9±0.5 ***	4.6±0.5 ***	5.0 ***	5.0 ***	3.9±0.6 ***	4.1±0.5 ***	
Diplotaxis tenuifolia	2.8±0.3 **	3.4±0.4 ***	3.9±0.5 ***	4.7±0.3 ***	4.4±0.5 ***	NT	3.9±0.6 ***	3.9±0.6 ***	
Eruca vesicaria subsp. *sativa*	3.8±0.8 ***	3.2±0.6 ***	3.3±0.4 ***	5.0 ***	4.4±0.4 ***	5.0 ***	4.5±0.4 ***	4.6±0.3 ***	
Raphanus sativus var. *sativus*	3.7±0.9 ***	**3.9±0.5 *****	**4.3±0.4 *****	5.0 ***	4.3±0.4 ***	**5.0 *****	**4.7±0.4 *****	4.8±0.3 ***	
Cardamineae									
Nasturtium officinale	3.4±0.5 ***	3.4±0.5 ***	3.5±0.3 ***	4.2±0.7 ***	2.2±0.8 **	4.0±0.5 ***	4.8±0.3 ***	1.5±0.6 **	Black spots were produced on lower leaves, then leaf blight with rich sporulation was observed at 7–10 dpi.

Table 5.5. (Continued).

Inoculated plants	Disease severity[a] and pathogenicity[b] by inoculation with:								Notes[c]
	Alternaria brassicae			*Alternaria brassicicola*			*Alternaria japonica*		
	AC29	MAFF 240791	AC62	MAFF 246772	MAFF 246773	AC70	MAFF 246775	AC96	
Eutremeae									Leaves inoculated with *Abe* showed distinct black spots with sporulation at 7 dpi. Leaves inoculated with *Aba* and *Aja* showed mostly indistinct tip burn with no to rare sporulation by 10 dpi.
Eutrema japonicum	1.5±0.6 *	3.0±1.6 ***	3.0 **	1.5±0.4 *	2.0±3.0 **	1.1±0.4 *	1.3±0.7 *	2.0 *	
Iberideae									Small black spots were produced, then the plant easily defoliated with sporulation by 10 dpi; relatively severe for *Aba*.
Iberis sempervirens	1.6±0.5 **	1.7±0.4 **	3.4±0.5 ***	4.8±0.2 ***	3.6±0.9 ***	2.8±0.6 **	1.9±0.4 **	2.0±0.4 **	
Lepidieae									No distinct symptoms were typically observed; *Aba* isolate AC70 and *Aja* isolate MAFF 246775 rarely produced necrotic spots without sporulation by 7 dpi.
Capsella bursa-pastoris	0	0	0	0	0	0.6±0.5 *	0.5±0.4 *	0	
Amaranthaceae									No distinct symptoms observed in this test over 14 dpi, even on *Spinacia* inoculated with *Aba* isolate MAFF 246773 ex *Spinacia*.
Beta vulgaris	0	0	0	0	0	NT	NT	NT	
Chenopodium giganteum	0	0	0	NT	NT	NT	NT	NT	
Spinacia oleracea	NT	NT	NT	0	**0**	0	NT	NT	
Apiaceae									
Daucus carota	NT	0	NT	0	0	NT	NT	NT	
Asteraceae									
Callistephus chinensis	NT	NT	NT	0	0.2±0.3	NT	NT	NT	
Lactuca sativa	0	0.3±0.2 *	0	0	0	NT	0.2±0.3	0	
Convolvulaceae									
Ipomoea nil	0	0	0.3±0.3 *	NT	NT	NT	NT	NT	
Cucurbitaceae									
Cucumis sativus	0.5±0.4 *	0	0	0	0	NT	NT	NT	
Cucurbita maxima	0.2±0.3 *	0	0	NT	NT	NT	NT	NT	
C. pepo	NT	NT	NT	0.2±0.2	0	NT	NT	NT	

Table 5.5. (Continued).

Inoculated plants	Disease severity[a] and pathogenicity[b] by inoculation with:								Notes[c]
	Alternaria brassicae			*Alternaria brassicicola*			*Alternaria japonica*		
	AC29	MAFF 240791	AC62	MAFF 246772	MAFF 246773	AC70	MAFF 246775	AC96	
Fabaceae									
Phaseolus vulgaris	NT	0	NT	0	0	NT	NT	NT	
Vicia faba	NT	0	NT	0	0	NT	NT	NT	
Vigna unguiculata subsp. *unguiculata*	NT	NT	NT	NT	NT	NT	0.2±0.3	0	
Onagraceae									
Clarkia amoena	NT	0	NT	0	0	NT	NT	NT	
Pedaliaceae									
Sesamum indicum	NT	NT	NT	0	0	NT	0.0	0	
Solanaceae									
Capsicum annuum	NT	0	NT	NT	NT	NT	NT	NT	
Solanum lycopersicum	NT	NT	NT	0.1±0.2	0.3±0.3	NT	NT	NT	
Poaceae									
Zea mays	NT	0	NT	NT	NT	NT	NT	NT	

[a] Mean disease severity at 7 d post-inoculation (dpi) rated on a 0–5 scale (0: no visible lesions, 1: <10 % leaf area affected, 2: 11–25 % leaf area affected, 3: 26–50 % leaf area affected, 4: 51–75 % leaf area affected, and 5: >75 % leaf area affected or defoliation). 95 % confidence intervals are also indicated. NT: not tested. Results for the original plant sources of each *Alternaria* isolate are indicated in bold.

[b] Pathogenicities were evaluated by the presence or absence of distinct lesions and sporulation on lesions, and are indicated with asterisks (***: strongly pathogenic, showing distinct lesions with rich sporulation; **: weakly pathogenic, showing indistinct or fewer distinct lesions with sporulation; *: weakly pathogenic to opportunistic, showing few, indistinct lesions with no to rare sporulation; blank: non-pathogenic, showing neither distinct lesions nor sporulation).

[c] Fungal names are abbreviated as follows: *Abe: Alternaria brassicae*, *Aba: A. brassicicola*, and *Aja*: *A. japonica*.

Fig. 5.7. Pathogenicity of *Alternaria brassicicola* (MAFF 246772). **A.** On *Brassica oleracea* var. *capitata* at 7 d post-inoculation (dpi). **B.** On *B. rapa* subsp. *chinensis* at 7 dpi. **C.** On *Raphanus* at 7 dpi. **D.** On *Eruca* at 7 dpi. **E.** On *Nasturtium* at 7 dpi. **F.** On *Iberis* at 7 dpi. **G.** On *Lobularia* at 18 dpi. **H.** On *Matthiola* at 7 dpi. **I.** On *Eutrema* at 18 dpi. **J.** On *Aubrieta* at 18 dpi. **K.** On *Capsella* at 7 dpi. **L.** On *Spinacia* at 7 dpi.

Three isolates of *A. brassicicola* (MAFF 246772, MAFF 246773, and AC70) were applied at an average of 3.2×10^6 conidia/mL (Table 5.5). Showing similar results to those of *A. brassicae*, three isolates of *A. brassicicola* had strong pathogenicity toward *Brassiceae* plants, *N. officinale*, *I. sempervirens*, *L. maritima*, and *M. incana* (Fig. 5.7A–H). On the other hand, inoculated *E. japonicum* leaves mostly showed only indistinct tip burn with no to rare sporulation by 18 dpi (Fig. 5.7I), and the inoculated leaves of *Aubrieta* sp. and *C. bursa-pastoris* showed no distinct symptoms. However, sporulation was often observed on suberized stem surfaces of the

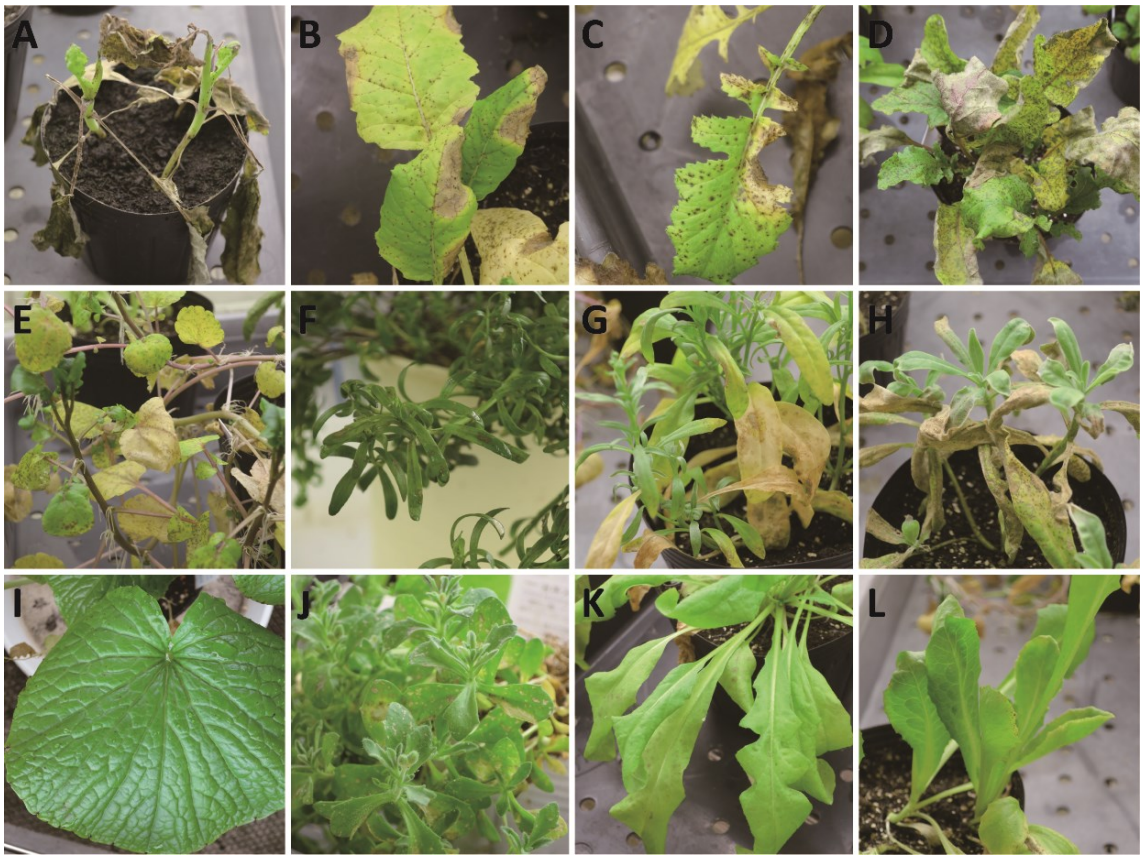

Fig. 5.8. Pathogenicity of *Alternaria japonica* (MAFF 246775). **A.** On *Brassica oleracea* var. *capitata* at 9 d post-inoculation (dpi). **B.** On *B. rapa* subsp. *rapa* at 10 dpi. **C.** On *Raphanus* at 10 dpi. **D.** On *Eruca* at 4 dpi. **E.** On *Nasturtium* at 4 dpi. **F.** On *Iberis* at 4 dpi. **G.** On *Lobularia* at 9 dpi. **H.** On *Matthiola* at 9 dpi. **I.** On *Eutrema* at 7 dpi. **J.** On *Aubrieta* at 7 dpi. **K.** On *Capsella* at 10 dpi. **L.** On *Lactuca* at 9 dpi.

former species at 7 dpi, and rarely produced necrotic spots on leaves of the latter without sporulation by 7 dpi (Fig. 5.7J, K). No distinct symptoms appeared on the non-*Brassicaceae* plants, even on *Spinacia*, which was the original source of MAFF 246773 (Fig. 5.7L).

Two isolates of *A. japonica* (MAFF 246775 and AC96) were applied at an average of 1.7×10^6 conidia/mL (Table 5.5). Similar results as those observed on the former two species were obtained on *Brassiceae* plants, *N. officinale*, *I. sempervirens*, *L. maritima*, and *M. incana* (Fig. 5.8A–H). The inoculated leaves of *E. japonicum*, *Aubrieta* sp., and *C. bursa-pastoris* showed no

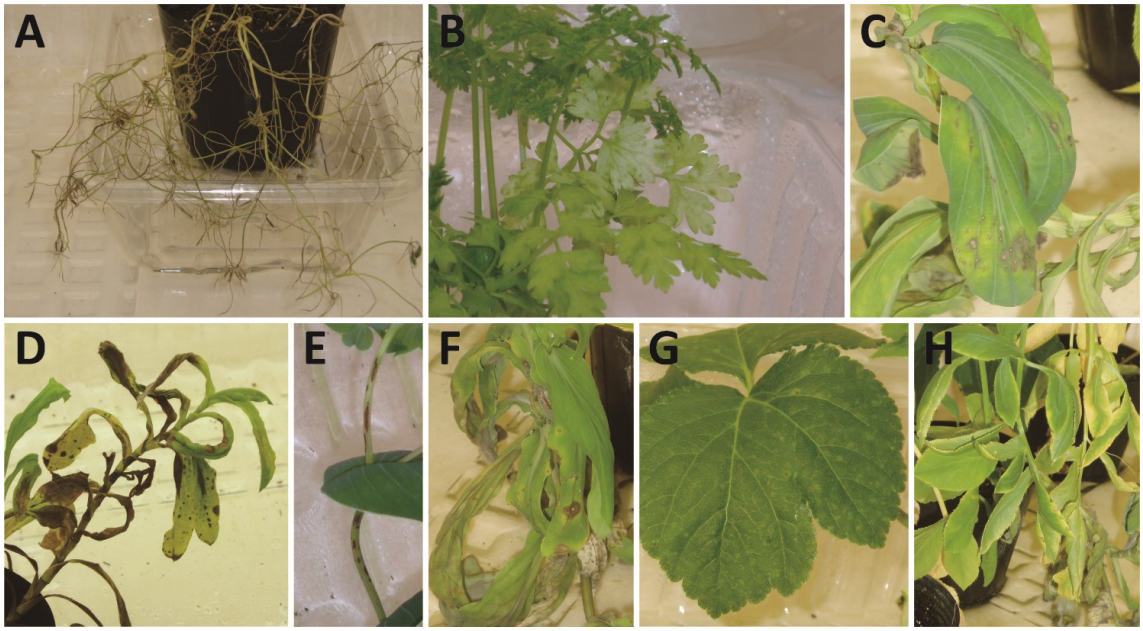

Fig. 5.9. Pathogenicity of two *Alternaria* species on *Apiaceae*. **A, B.** *Alternaria cumini* (MAFF 246774); **A.** On *Cuminum* at 7 d post-inoculation (dpi). **B.** On *Anthriscus* at 7 dpi. **C–H.** *Alternaria* sp. (MAFF 246776); **C, D.** On *Bupleurum* at 8 dpi. **E.** On stem of *Bupleurum* at 8 dpi. **F.** On *Bupleurum* at 11 dpi. **G.** On *Angelica* at 10 dpi. **H.** On *Ammi* at 11 dpi.

distinct symptoms, though necrotic spots without sporulation were rarely produced at 14 dpi (Fig. 5.8I–K). No distinct symptoms appeared on the non-*Brassicaceae* plants (Fig. 5.8L).

Alternaria cumini *and a novel species infecting* Apiaceae

An isolate of *A. cumini* (MAFF 246774) was applied at an average of 5.0×10^4 conidia/mL (Table 5.6). Leaf spots on *Cuminum cyminum*, which was the original source of the fungus used for inoculation, appeared at 2 dpi, then leaves became severely blighted and rotten with rich sporulation within 6 dpi (Fig. 5.9A). On the inoculated leaves of *Petroselinum crispum* and *Anthriscus cerefolium*, mostly small spots or tip burn appeared at 7 dpi, but with rare sporulation within 30 dpi (Fig. 5.9B). No distinct symptoms were observed on the other seven apiaceous plants, including *Coriandrum sativum* and *Daucus carota* at 30 dpi.

Table 5.6. Experimental host ranges of *Alternaria* species infecting *Apiaceae*.

Inoculated plants	Disease severity[a] and pathogenicity[b] by inoculation with:			References of host range of the other species on *Daucus carota*			Notes[f]
	Alternaria cumini	Novel species	*Alternaria petroselini*[c]	*Alternaria carotiincultae*[d]	*Alternaria dauci*[e]	*Alternaria radicina*[d]	
	MAFF 246774	MAFF 246776	MAFF 243057				
Apiaceae, *Apioideae*							
Apieae							
Ammi majus	NT	0.1±0.2	2.9±1.1 ***	NT	NT	NT	Small spots appeared on leaves of *Petroselinum* inoculated with *Acu*, but no sporulation was observed by 30 dpi. No distinct symptoms were observed on leaves inoculated with MAFF 246776 by 30 dpi. *Ape* and the other three species are widely and partially pathogenic to the tribe, respectively. The host range of *Aca* and *Ara* is identical within the tribe.
Anethum graveolens	NT	NT	4.4±0.7 ***	Weak–Non	HE	Weak–Non	
Apium graveolens	0.1±0.2	0.3±0.3	3.2±1.2 ***	Weak	L	Weak	
Foeniculum vulgare	NT	NT	3.3±1.1 ***	Weak	HE	Weak	
Petroselinum crispum	0.3±0.3 *	0	**3.8±0.7 *** **	Non	L	Non	
Bupleureae							Leaves inoculated with MAFF 246776 showed distinct black spots with sporulation within 7 dpi. *Ape* is also weakly pathogenic.
Bupleurum rotundifolium	0	**3.9±0.7 *** **	2.6±1.1 **	NT	NT	NT	
Careae							No distinct symptoms were observed on leaves inoculated with *Acu* and MAFF 246776, and no other pathogenic species were found.
Carum carvi	0.3±0.6	0.2±0.2	0	Weak–Non	NT	Weak–Non	
Coriandreae							No distinct symptoms were observed on the inoculated leaves of the two examined species, while *Ape*, *Aca*, *Ada*, and *Ara* were shown to be pathogenic.
Coriandrum sativum	0	0	3.5±0.7 ***	Weak	Hn	Weak	
Oenantheae							No distinct symptoms were observed on the inoculated leaves.
Cryptotaenia japonica	0	0	0	NT	NT	NT	
Scandiceae							
Daucinae							*Cuminum* leaves inoculated with *Acu* showed severe leaf blight with rich sporulation at 5 dpi, and *Daucus* leaves inoculated with examined three species showed no distinct symptoms at 30 dpi.
Cuminum cyminum	**4.5±0.3 *** **	NT	5.0 ***	NT	NT	NT	
Daucus carota	0	0	0.6±0.3 *	**HP**	**HE**	**HP**	
Scandicinae							Small spots and tip burn without sporulation was rarely observed on leaves inoculated with *Acu* at 14 dpi, while *Ape* and *Ada* are clearly pathogenic.
Anthriscus cerefolium	0.4±0.4 *	NT	3.7±0.7 ***	NT	Hn	NT	

Table 5.6. (Continued).

Inoculated plants	Disease severity[a] and pathogenicity[b] by inoculation with:			References of host range of the other species on *Daucus carota*			Notes[f]
	Alternaria cumini MAFF 246774	Novel species MAFF 246776	*Alternaria petroselini*[c] MAFF 243057	*Alternaria carotiincultae*[d]	*Alternaria dauci*[e]	*Alternaria radicina*[d]	
Selineae							
Angelica keiskei	0	0.7±1.3 *	2.2±1.0 **	NT	NT	NT	Small necrotic spots without sporulation were observed at 30 dpi on leaves inoculated with MAFF 246776. Only *Ape* is weakly pathogenic.

[a] Mean disease severity at 7 d post-inoculation (dpi) rated on a 0–5 scale (0: no visible lesions, 1: <10 % leaf area affected, 2: 11–25 % leaf area affected, 3: 26–50 % leaf area affected, 4: 51–75 % leaf area affected, and 5: >75 % leaf area affected or defoliation). 95 % confidence intervals are also indicated. NT: not tested. Results for the original plant sources of each *Alternaria* isolate are indicated in bold.

[b] Pathogenicities were evaluated by the presence or absence of distinct lesions and sporulation on lesions, and are indicated with asterisks (***: strongly pathogenic, showing distinct lesions with rich sporulation; **: weakly pathogenic, showing indistinct or fewer distinct lesions with sporulation; *: weakly pathogenic to opportunistic, showing few, indistinct lesions with no to rare sporulation; blank: non-pathogenic, showing neither distinct lesions nor sporulation).

[c] From results reported by Nishikawa & Nakashima (2013).

[d] From results reported by Pryor & Gilbertson (2002). HP: highly pathogenic, Weak: weakly pathogenic, Non: non-pathogenic. They also determined that *A. carotiincultae*, *A. petroselini*, and *A. radicina* were weakly or non-pathogenic to *Pimpinella anisum* (tribe *Pimpinelleae*) and *Pastinaca sativa* (tribe *Tordylieae*).

[e] From results reported by Boedo *et al.* (2012). HE: species exhibiting a relatively high disease index with expanding lesions, Hn: species showing a relatively high disease index with non-expanding lesions, L: species exhibiting a relatively low disease index with non-expanding lesions. They also noted that the inoculated leaves of *Pastinaca* and non-*Apiaceae* plants (corn salad and leek) showed a low disease index or were symptomless.

[f] Fungal names are abbreviated as follows: *Acu*: *Alternaria cumini*, *Ape*: *A. petroselini*, and *Aca*: *A. carotiincultae*, *Ada*: *A. dauci*, *Ara*: *A. radicina*.

A conidial suspension of a novel ex *Bupleurum rotundifolium* isolate (MAFF 246776) was applied at an average of 6.4 × 10^4 conidia/mL (Table 5.6). The inoculated leaves of *B. rotundifolium* showed distinct black spots within 5 dpi, and sporulation was abundant on lesions at 7 dpi (Fig. 5.9C–F). Small necrotic spots without sporulation were sometimes observed within 30 dpi on the inoculated leaves of *Angelica keiskei* (Fig. 5.9G). No distinct symptoms were observed on the inoculated leaves of the other seven plants at 30 dpi (Fig. 5.9H).

Fig. 5.10. Pathogenicity of *Alternaria* sp. ex *Petunia* (MAFF 246770). **A–C.** On *Petunia*; **A, B.** at 3 d post-inoculation (dpi). **C.** at 7 dpi. **D.** On *Solanum lycopersicum* at 6 dpi. **E.** On *S. melongena* at 7 dpi. **F.** On *Brugmansia* at 9 dpi.

Table 5.7. Experimental host ranges of *Alternaria* species on *Solanaceae*.

Inoculated plants	Disease severity[a] and pathogenicity[b] by inoculation with:		References of host range of *Alternaria solani*[d]	Notes
	Novel species	*Alternaria crassa*[c]		
	MAFF 246770	MAFF 243056		
Solanaceae				
Nicotianoideae				No distinct symptoms were observed.
Nicotiana tabacum	0.3±0.4	0	Non	
Petunioideae				Severe necrosis appeared on leaves inoculated with MAFF 246770 at 2 dpi, which then became rotten with rich sporulation.
Petunia × atkinsiana	**5.0 *****	0.2±0.3 *	NT	
Solanoideae				Small necrotic spots were observed on *Solanum* inoculated with MAFF 246770 at 2 dpi, which occasionally became slightly expanded with sporulation until 7 dpi. MAFF 246770 showed no pathogenicity to the other examined *Solanoideae* plants. Host selectivities of *A. crassa* and *A. solani* are reported as to each original source plant and *Capsicum*.
Capsiceae				
Capsicum annuum	0	4.2±0.7 ***	M	
Datureae				
Brugmansia × candida	0	**2.3±0.7 *****	Non	
Physaleae				
Physalis alkekengi var. *franchetii*	0	0.3±0.4	NT	
Solaneae				
Solanum lycopersicum	2.4±0.7 *	1.2±0.3 *	**S**	
S. melongena	2.9±0.9 *	0.4±0.4	**S**	
Fabaceae				No distinct symptoms were observed over 30 dpi on leaves of non-host plants inoculated with MAFF 246770.
Vigna unguiculata	0	NT	NT	
Poaceae				
Zea mays	0	NT	NT	

[a] Mean disease severity at 7 d post-inoculation (dpi) rated on a 0–5 scale (0: no visible lesions, 1: <10 % leaf area affected, 2: 11–25 % leaf area affected, 3: 26–50 % leaf area affected, 4: 51–75 % leaf area affected, and 5: >75 % leaf area affected or defoliation). 95 % confidence intervals are also indicated. NT: not tested. Results for the original source plant species (or close relatives) of each *Alternaria* species are indicated in bold.

[b] Pathogenicities were evaluated by the presence or absence of distinct lesions and sporulation on lesions, and are indicated with asterisks (***: strongly pathogenic, showing distinct lesions with rich sporulation; **: weakly pathogenic, showing indistinct or fewer distinct lesions with sporulation; *: weakly pathogenic to opportunistic, showing few, indistinct lesions with no to rare sporulation; blank: non-pathogenic, showing neither distinct lesions nor sporulation).

[c] From results reported by Nishikawa & Nakashima (2013).

[d] From results reported by Cardoso (2014). S: susceptible, M: caused mild symptoms, Non: non-pathogenic (symptoms or pathogen structures absent). Cardoso's results also confirmed virulences of *A*. *solani* toward three asteraceous species (*Ageratum conyzoides*, *Erigeron bonariensis*, and *Galinsoga parviflora*), and *Rumex acetosa* (*Polygonaceae*).

***A novel species ex* Petunia *infecting* Solanaceae**

A conidial suspension of a novel ex *Petunia* × *atkinsiana* isolate (MAFF 246770) was applied at an average of 8.2×10^5 conidia/mL (Table 5.7). Irregular-shaped necrosises appeared abundantly on the inoculated leaves at 2 dpi, and then lesions quickly expanded and caused severe rot of the whole plants with rich sporulation (Fig. 5.10A–C). Small necrotic spots were observed on the inoculated leaves of *Solanum lycopersicum* and *S. melongena* at 2 dpi, and were slightly expanded with little sporulation within 7 dpi (Fig. 5.10D, E). No distinct symptoms were observed on the other three *Solanoideae* plants, *Nicotiana tabacum*, and two

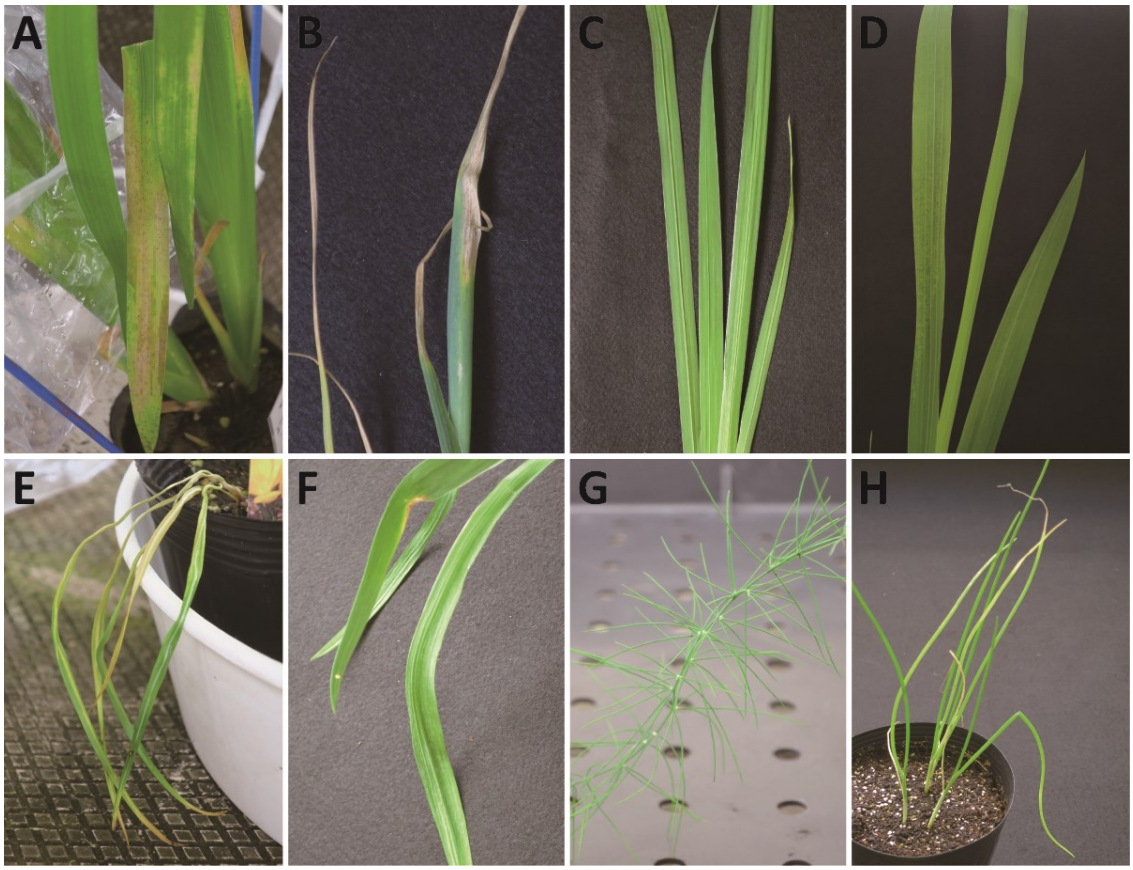

Fig. 5.11. Pathogenicity of *Alternaria iridicola* (MAFF 246890). **A.** On *Iris laevigata* at 6 post-inoculation (dpi). **B.** On *Iris* × *hollandica* at 14 dpi. **C.** On *I. ensata* var. *spontanea* at 14 dpi. **D.** On *Gladiolus* at 14 dpi. **E.** On *Crocus* at 6 dpi. **F.** On *Freesia* at 14 dpi. **G.** On *Asparagus* at 14 dpi. **H.** On *Allium* at 14 dpi.

Table 5.8. Experimental host range of *Alternaria iridicola*.

Inoculated plants	Disease severity[a] and pathogenicity[b] by inoculation with:		Notes
	MAFF 246890	MAFF 246771	
Amaryllidaceae			Small yellow spots and tip burn without sporulation were rarely observed at 14 dpi.
Allium fistulosum	0.7±0.7 *	0.3±0.3 *	
Asparagaceae			No distinct symptoms observed by 14 dpi.
Asparagus officinalis	0.1±0.3	NT	
Iridaceae			
Crocoideae			Slightly yellowing on tips with occasional sporulation were observed.
Crocus sp.	1.0±0.8 *	1.7±0.7 *	
Iridoideae			Leaf spots and sever blight with rich sporulation on *I. laevigata* and *Iris* × *hollandica* were observed at 7 dpi, but *I. ensata* var. *spontanea* never showed any symptoms over 14 dpi.
Iris ensata var. *spontanea*	**0.6±0.5**	**0.3±0.3**	
I. laevigata	**3.5±1.3** ***	**4.0±0.8** ***	
Iris × *hollandica*	**4.1±0.6** ***	**4.4±0.5** ***	
Ixioideae			Small yellow spots were often produced on *Gladiolus* by 14 dpi, but never expanded and sporulated. No distinct symptoms were observed on *Freesia* by 14 dpi.
Freesia refracta	0	0	
Gladiolus sp.	0.3±0.5	0.3±0.3	

[a] Mean disease severity at 7 d post-inoculation (dpi) rated on a 0–5 scale (0: no visible lesions, 1: <10 % leaf area affected, 2: 11–25 %, 3: 26–50 %, 4: 51–75 %, and 5: >75 % or defoliated). 95 % confidence intervals also indicated. NT: not tested. Results of the original source plant genus of each *Alternaria* species are indicated in bold.

[b] Pathogenicities were evaluated by presence or absence of distinct lesion and sporulation on lesion, and indicated asterisks (***: strongly pathogenic, showing distinct lesions with rich sporulation, **: weakly pathogenic, showing indistinct or fewer distinct lesions with sporulation, *: weakly pathogenic to opportunistic, showing fewer indistinct lesions with no to rare sporulation, blank: non-pathogenic, showing distinct lesions nor sporulation).

non-solanaceous plants (Fig. 5.10F).

Alternaria iridicola *infecting* Iridaceae

Two isolates of *A. iridicola* (MAFF 246890 and MAFF 246771) were applied at an average of 3.2 × 10^5 conidia/mL, and both isolates showed similar results (Table 5.8). Distinct leaf spots appeared on *Iris* spp., except for *I. ensata* var. *spontanea*, at 7 dpi, and then the inoculated leaves became severely blighted with rich sporulation (Fig. 5.11A, B). As for *I. ensata* var. *spontanea*, neither distinct symptoms nor sporulation were observed within 14 dpi (Fig. 5.11C). Small yellow spots and slightly yellowing spots with no or poor sporulation were observed at 7 dpi on *Gladiolus* sp. and *Crocus* sp., respectively (Fig. 5.11D, E). No distinct symptoms were observed on *Freesia refracta* and *Asparagus officinalis*, while yellow spots and tip burn without sporulation were sometimes observed on leaves of *Allium fistulosum* at 14 dpi (Fig. 5.11F–H).

Alternaria porri *on non-host plants*

Obtained *A. porri* isolates from non-host plants, such as isolates AC2 ex *Viola* × *wittrockiana* (*Violaceae*), AC6 ex *Calibrachoa* sp. (*Solanaceae*), and AC35 ex *Eustoma exaltatum* subsp. *russellianum* (*Gentianaceae*), were used to inoculate each original source plant by spraying with conidial suspension concentrated at an average of 2.0 × 10^5 conidia/mL. No isolates showing pathogenicity toward each source plant were found, but not to leaves of *Allium fistulosum* (data not shown).

Taxonomy

Eighty five of the Japanese *Alternaria* isolates collected and examined consisted of 26 species—including three new species of the genus *Alternaria*—as characterized based on integrated species criteria. Each taxon is described for each *Alternaria* section in alphabetical order as follows.

Section *Alternantherae* D.P. Lawr., Gannibal, Peever & B.M. Pryor, *Mycologia* **105**: 540. 2013. [MB 802305].

Four species are recognized in this section (Lawrence *et al.* 2013; Woudenberg *et al.* 2013), and Gannibal (2018) added two species thereafter. All the species and phylogenetically uncertained species (*A*. *crassoides* and *A*. *pimpriana*) were former members of the genus *Nimbya* and parasitic toward *Amaranthaceae* plants (Simmons 1989, 1995b, 2004). Among these, two existing species and one novel species were described, with phylogenetic analyses were conducted in the present study.

Alternaria celosiicola Jun. Nishikawa & C. Nakash., *J. Phytopathol*. **161**: 606. 2013. [MB 800983]. **Figs** 4.2; 4.3a; **5.12**.

≡ *Nimbya celosiae* E.G. Simmons & Holcomb, *Mycotaxon* **55**: 144. 1995. [MB 413578]

≡ *Alternaria celosiae* (E.G. Simmons & Holcomb) Lawrence, Park & Pryor, *Mycol. Progr.* **11**: 811. 2012, nom. illeg. (ICN Art. 53.1). non *Alternaria celosiae* (Tassi) O. Săvul., *Herb. Mycol. Rom*.: fasc. **30**, no. 1489. 1950. [MB 807924].

≡ *Alternaria cristata* D.P. Lawr., M.S. Park & B.M. Pryor, *Mycol. Progr.* **13** (2): 259. 2014, nom. nud. [MB 803181].

Type: **USA**, Louisiana, on *Celosia cristata* L., Jul. 1993, *G.E. Holcomb*, holotype BPI 803020 [MBT 83692], isotype IMI 369150 and IMI 369153, culture ex-type EGS42.013.

Collection examined: **Japan**, Kanagawa Prefecture, Fujisawa, on leaves of *Celosia argentea* L. var. *plumosa* Voss, 29 Jun. 2006, *S. Masugi & Y. Makizumi*, MUMH 11676 and MUMH 11701, living culture MAFF 243058 = AC28.

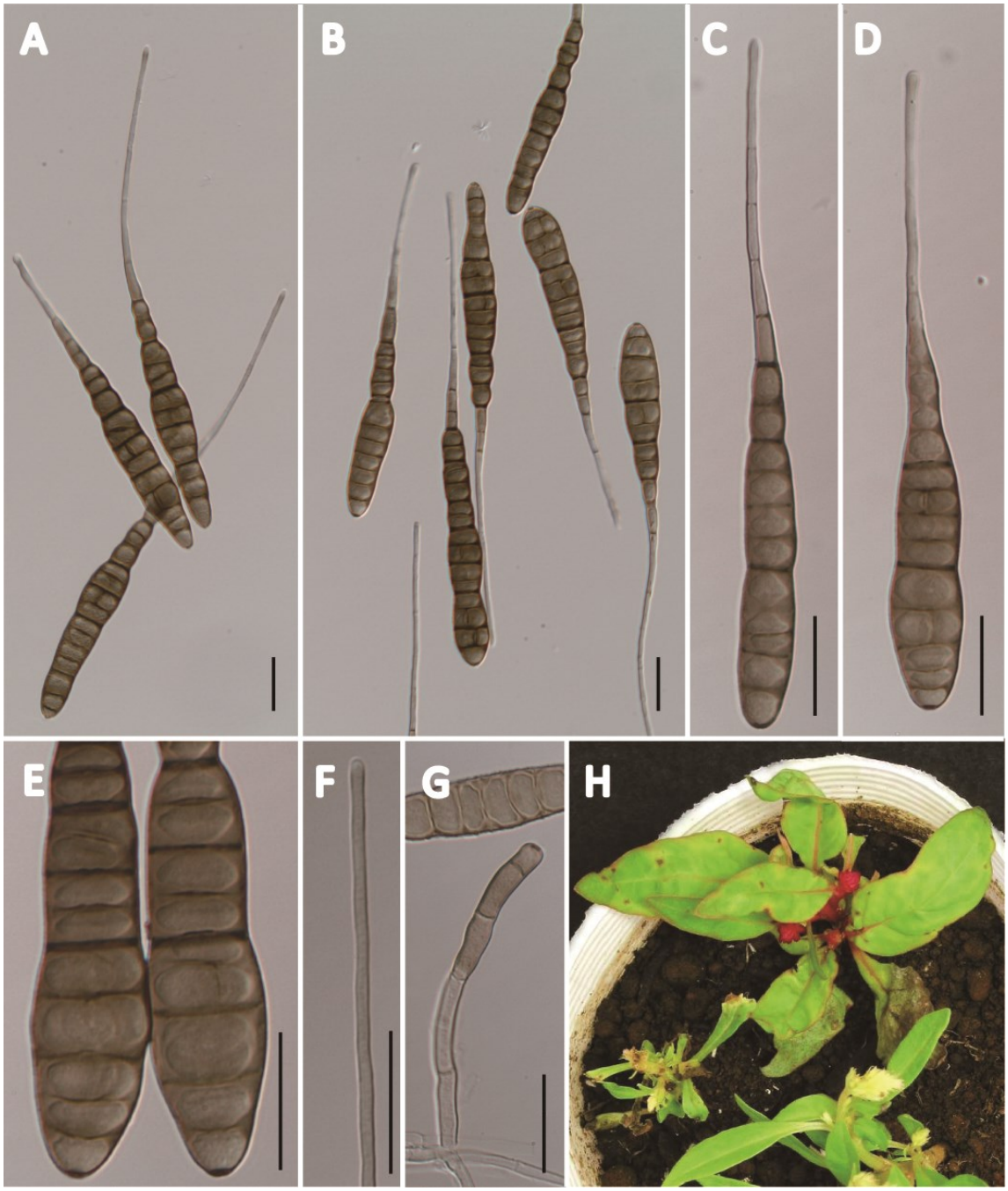

Fig. 5.12. Morphological features of Japanese isolates of *Alternaria celosiicola* (MAFF 243058) on potato-carrot agar medium. **A–E.** Conidia and lumina. **F.** Colored beak. **G.** Conidiophores. **H.** Natural symptoms on *Celosia argentea* var. *plumosa*. Bars = 25 µm.

Morphological character on V8 medium: Conidia are usually solitary, pale brown to brown, subcylindrical to narrowly ellipsoid, 42–755 µm in total length, and their surface is commonly smooth; conidial bodies measure 42–180 × 10–26 µm, with 2–17 transverse and 0–5 longitudinal septa, mostly consisting of distosepta, constricted at some transverse septa. The lumina are usually distinct and octagonal to round; beaks are filamentous to columnar and are straight to slightly curved, subhyaline to pale brown, multiseptate, unbranched, sometimes knobbed at the apex, and measure 49–575 × 2–4 µm. Conidiophores are short and broad, measuring 25–69 × 5–7 µm. Morphology observed on PCA medium or lesion were similar to those observed on V8 medium (Table 5.3).

Culture characteristics on PDA medium: Colonies are fast-growing, reaching 71.2 ± 0.9 mm in diam after 7 d at 25 °C, and are rounded with white margins at the circumference; aerial hypha are cottony, pale green to grayish green, reverse center black to dark green; sporulation is sparse; no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Celosia* (*Amaranthaceae*).

Symptoms: Leaf spots on *C. cristata* are circular to subcircular, 2–10 mm in diam, brown to dark brown with distinct reddish margin, and often surrounded by a yellowish halo, becoming confluent.

Experimental host range: Pathogenic to *C. cristata*, *Amaranthus tricolor*, and *Gomphrena globosa*, but not to *Beta vulgaris* and *Spinacia oleracea* (Tables 4.1; 5.4).

Distribution: USA (Simmons 1995b), China (Zhao & Zhang 2005), and Japan (Nishikawa & Nakashima 2013).

Distinctive features: Conidia are larger than those of other related species in sect. *Alternantherae*; conidial bodies usually exceeded 100 × 20 µm, and beaks exceed 200 µm long. Conidial bodies commonly consist of a distosepta-like internal wall structure with octagonal

lumina. This species is widely pathogenic not only to *Celosia*, but also to *Amaranthus*, *Alternanthera*, and *Gomphrena*, and is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Notes: Lawrence *et al*. (2012) transferred this species from the genus *Nimbya* to *Alternaria* based on phylogenetic analysis, but the proposed name—*A*. *celosiae* (E.G. Simmons & Holcomb) Lawrence, Park & Pryor—resulted in a later homonym of *A*. *celosiae* (Tassi) O. Săvul. Although Lawrence *et al.* (2014) renamed the epithet of *A*. *celosiae* to *Alternaria cristata* Lawrence, Park, & Pryor (MB 803181) without detailed descriptions, this name is a later synonym of *A*. *celosiicola* described by Nishikawa & Nakashima (2013).

Alternaria gomphrenae Togashi, *Bull. Imp. Coll. Agric. Forest. Morioka, Japan* **9**: 6. 1926. [MB 266214]. **Figs 5.13**, **14**.

≡ *Nimbya gomphrenae* (Togashi) E.G. Simmons, *Sydowia* **41**: 324. 1989. [MB 125920].

= *Pseudocercospora gomphrenicola* Chidd., *Sci. Cult.* (*Calcutta*) **22**: 511. 1957. [MB 304346].

Type: **Japan**, Kyoto Prefecture, Kitashirakawa, on leaves of *Gomphrena globosa* L. (not specified; syntype specimens were assigned as lectotype and paralectotype, respectively, in this study) [MBT 179010].

Lectotype (**designated here**): **Japan**, Kyoto Prefecture, Kitashirakawa, on leaves of *G. globosa*, 24 Aug. 1924, *K. Togashi*, TNS-F-243868 [MBT 385025]; *Paralectotype*: **Japan**, Kyoto Prefecture, Kitashirakawa, on leaves of *G. globosa*, 5 Aug. 1925, *K. Togashi*, TNS-F-243861; ibid., 10 Aug. 1925, *K. Togashi*, TNS-F-243862; ibid., 4 Dec. 1925, *K. Togashi*, TNS-F-243866; ibid., 19 Aug. 1924, *Togashi*, TNS-F-243867; ibid., 22 Jun. 1925, *Togashi*, TNS-F-243872; ibid., 7 Aug. 1924, *T. Hemmi & K. Togashi*, TNS-F-243873; ibid., 17 Aug. 1924, *K. Togashi*, TNS-F-243875.

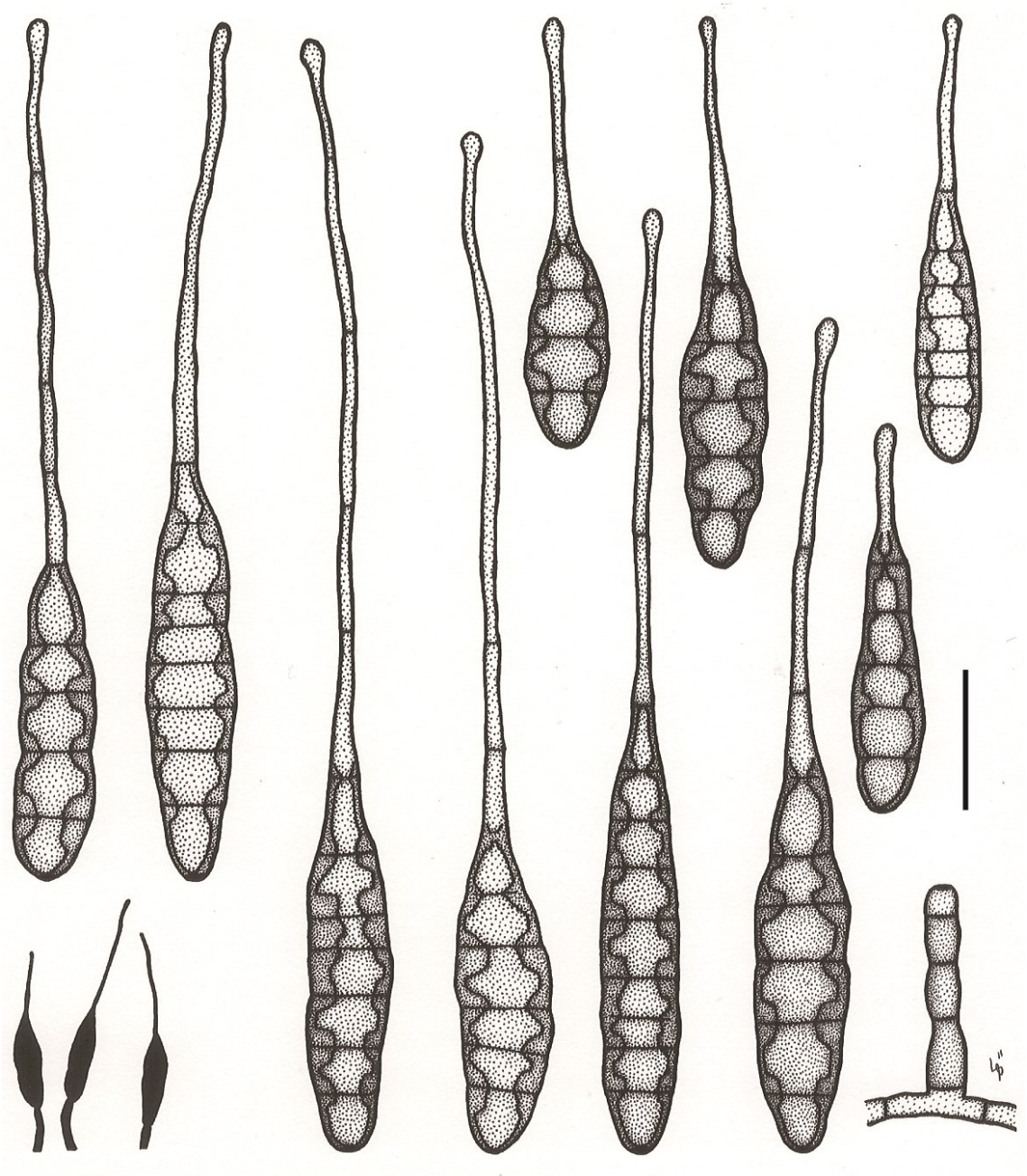

Fig. 5.13. Illustrations of *Alternaria gomphrenae* (MAFF 246769). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on V8 juice agar medium. Bar = 25 µm.

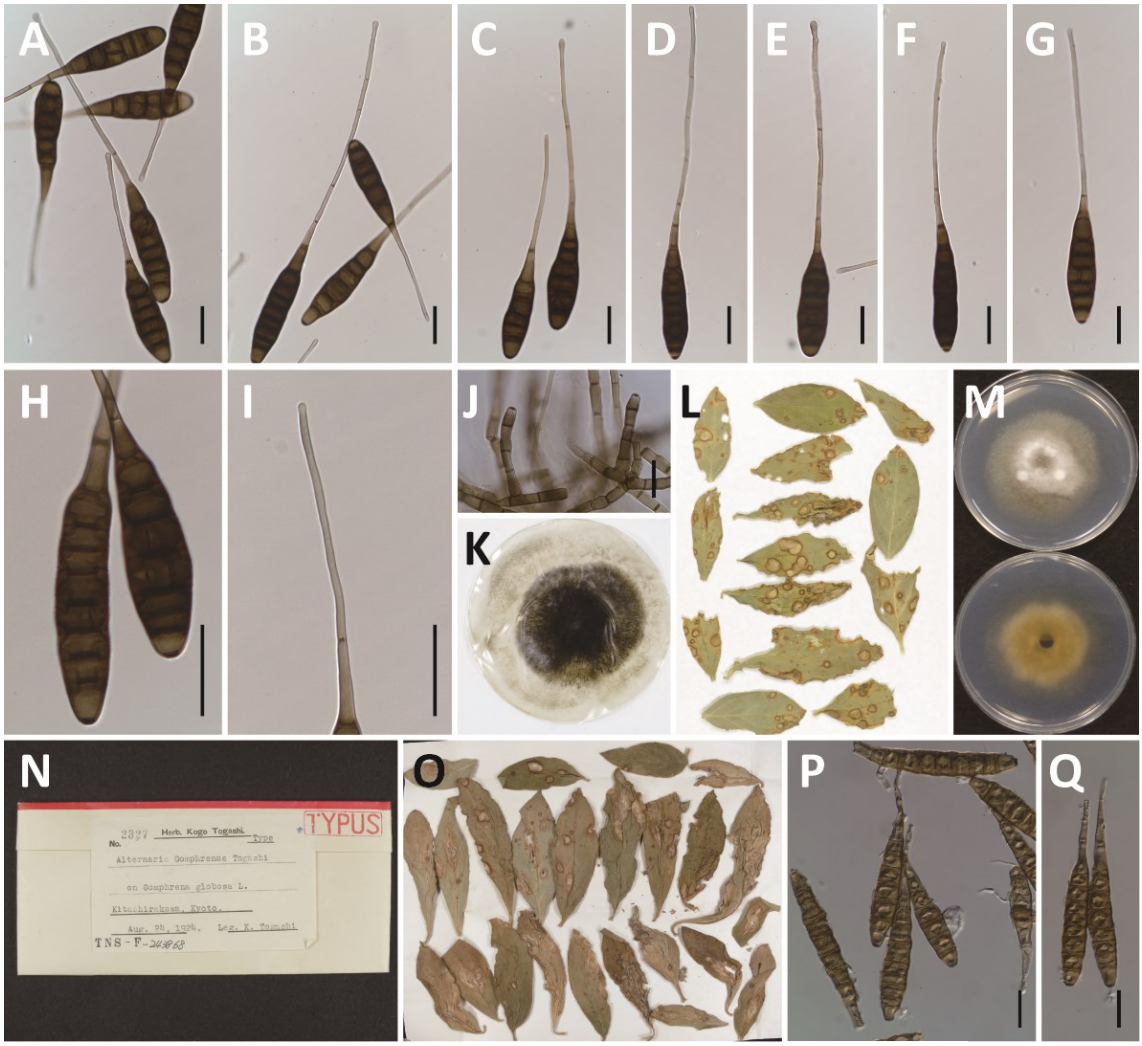

Fig. 5.14. Morphological features of Japanese isolates of *Alternaria gomphrenae* (MAFF 246769) on V8 juice agar medium. **A–H.** Conidia and lumina. **I.** Colored beak. **J.** Conidiophores. **K.** Dried culture specimen ex MAFF 246769 (epitype: TNS-F-85451). **L.** Natural symptoms on the specimen of *Gomphrena globosa* (isoepitype: MUMH 11685). **M.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). **N–O.** Lectotype specimen, TNS-F-243868. **P–Q.** Conidia on lectotype.

Epitype (**designated here**): **Japan**, Shizuoka Prefecture, Kakegawa, on leaves of *G. globosa*, 16 Oct. 2011, *J. Nishikawa*, TNS-F-85451 (dried culture of MAFF 246769) [MBT 385026],

isoepitype MUMH 11685, culture ex-epitype MAFF 246769 = AC81.

Collections examined: **Japan**, Shizuoka Prefecture, Kakegawa, on leaves of *G. globosa*, 16 Oct. 2011, *J. Nishikawa*, TNS-F-85451 (**epitype**), MUMH 11685 (**isoepitype**), living culture MAFF 246769 = AC81.

Additional specimens examined: **Japan**, Kyoto Prefecture, Kitashirakawa, on leaves of *Gomphrena globosa* L., 5 Aug. 1925, *K. Togashi*, TNS-F-243861; ibid., 10 Aug. 1925, *K. Togashi*, TNS-F-243862; ibid., 4 Dec. 1925, *K. Togashi*, TNS-F-243866; ibid., 19 Aug. 1924, *K. Togashi*, TNS-F-243867; ibid., 24 Aug. 1924, *K. Togashi*, TNS-F-243868 (**lectotype**); ibid., 22 Jun. 1925, *K. Togashi*, TNS-F-243872; ibid., 7 Aug. 1924, *T. Hemmi & K. Togashi*, TNS-F-243873; ibid., 17 Aug. 1924, *K. Togashi*, TNS-F-243875.

Morphological character on V8 medium: Conidia are usually solitary, brown to dark brown, subcylindrical to long obclavate, and 50–287 µm in total length, with a surface smooth; conidial bodies measure 35–77 × 10–17 µm, with 3–9 transverse and 0–1 longitudinal septa consisting of distosepta. The lumina are usually distinct, and are octagonal to round; filamentous beaks are usually straight, subhyaline to pale brown, sometimes multiseptated, unbranched, conspicuously border the conidial body, are often knobbed at the apex, and measure 13–216 × 2–4 µm. Conidiophores short and broad, measuring 25–65 µm × 5–8 µm. Conidial bodies on lectotype specimens (TNS-F-243868) measure 46–94 × 10–16 µm, with 4–10 transverse and no longitudinal septa. Morphology on PCA medium and lesions were similar to those observed on V8 medium (Table 5.3).

Culture characteristics on PDA medium: Colonies are slow to moderate-growing, reaching an average of 48 ± 1.8 mm in diam after 7 d at 25 °C. They are almost rounded, with inconspicuous margins at the circumference; aerial hypha are cottony, grayish green to white, reverse center pigmented with pale brown to reddish orange; sporulation is sparse.

Teleomorph: Not observed on RSA medium.

Natural host: *Gomphrena* (*Amaranthaceae*).

Symptoms: Leaf and stem spots on *G*. *globosa* are circular to elliptical, 2–10 mm in diam, pale brown with reddish margins, and become enlarged and confluent, resulting in leaf blighting.

Experimental host range: Selectively pathogenic to *Gomphrena*, but not to *Celosia* and *Amaranthus* in the inoculation tests conducted (Table 5.4).

Distribution: In Asia (Japan, Indonesia, China, etc.), as well as North and Latin America (USA, Cuba, Jamaica, etc.) (Yoshii 1933; Ellis 1976; Simmons 1989; Zhao & Zhang 2005).

Distinctive features: Conidia are long obclavate, shorter, and narrower (usually not exceeding 100 × 20 µm) than other species infecting *Amaranthaceae*. They rarely have longitudinal septa, with octagonal lumina and colored beaks. This species is selectively pathogenic to *G*. *globosa*, and recognizable phylogenetically via its ITS sequence (Fig. 5.3).

Notes: This species is the causal pathogen of leaf spot on *G. globosa*, and was first described by Togashi (1926) in Japan. In this description, Togashi also described ten specimens, but did not specify the holotype. Fortunately, eight of these specimens were preserved as 'TYPUS' in TNS; therefore, we re-examined these syntype specimens and selected TNS-F-243868 as a lectotype in the present study. However, we confirmed rich sporulation of *A*. *gomphrenae*, often together with those of a small-spored *Alternaria* species, and found that Togashi, in fact, failed to establish pure cultures, much less complete inoculation testing (Togashi 1926; Yoshii 1933; Simmons 1989, 1995b). Therefore, we also designated an epitype specimen, and provided the ex-epitype isolate here.

Alternaria paragomphrenae Jun. Nishikawa & C. Nakash., ***sp. nov.*** MycoBank MB 829109. **Figs 5.15**, **16**.

Diagnosis: Conidial bodies are cylindrical, commonly less than 100 µm in length and exceeded 20 µm in width, sometimes with longitudinal septa forming octagonal lumina, with colored beaks. Pathogenicity of the species is selective to *Gomphrena* and *Alternanthera*. It is phylogenetically recognizable among sect. *Alternantherae* via its *act*, *Alt a 1*, *gapdh*, *rpb2*, and *tef1* sequences.

Etymology: Named because of its close resemblance to *Alternaria gomphrenae*, both in conidial morphology and host range.

Descriptions: Leaf and stem spots appear on *Gomphrena haageana* (*Amaranthaceae*), and are pale brown with a small, grayish eye in the center surrounded by reddish margins. They are circular to elliptical, 2–6 mm in diam, scattered, show water-soaked enlargement, and become confluent resulting in leaf blighting. When grown on V8 medium, conidia are usually solitary, pale to brown, ellipsoid to cylindrical, and 58–409 µm in total length, with a smooth surface; conidial bodies measure 48–98 × 17–33 µm, with 3–7 transverse and 0–4 longitudinal septa consisting of distosepta, constricted at each transverse septa. The lumina range from distinct to indistinct, octagonal to round; filamentous beaks may be straight to curved, subhyaline to pale brown, sometimes multiseptated, unbranched, conspicuously border the conidial body, measure 25–316 × 3–5 µm, and are elongated on cultures rather than on lesions. Conidiophores are short and thick, measuring 27–81 × 5–7 µm. Morphology on PCA was similar to that observed on V8 medium; 60–294 µm in total length, conidial bodies measured 60–111 × 15–25 µm, with 2–9 transverse and 0–3 longitudinal septa, beaks measuring 14–208 × 2–5 µm, conidiophores measuring 43–125 × 6–9 µm. Conidia on lesions measure 35–173 µm in total length, and conidial bodies measure 25–99 × 8–26 µm, with 1–9 transverse and 0–2 longitudinal septa. Beaks measure 14–87 × 3–4 µm, and conidiophores 26–99 × 5–7 µm.

Fig. 5.15. Illustrations of *Alternaria paragomphrenae* (MAFF 246768). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on V8 juice agar medium. Bar = 25 µm.

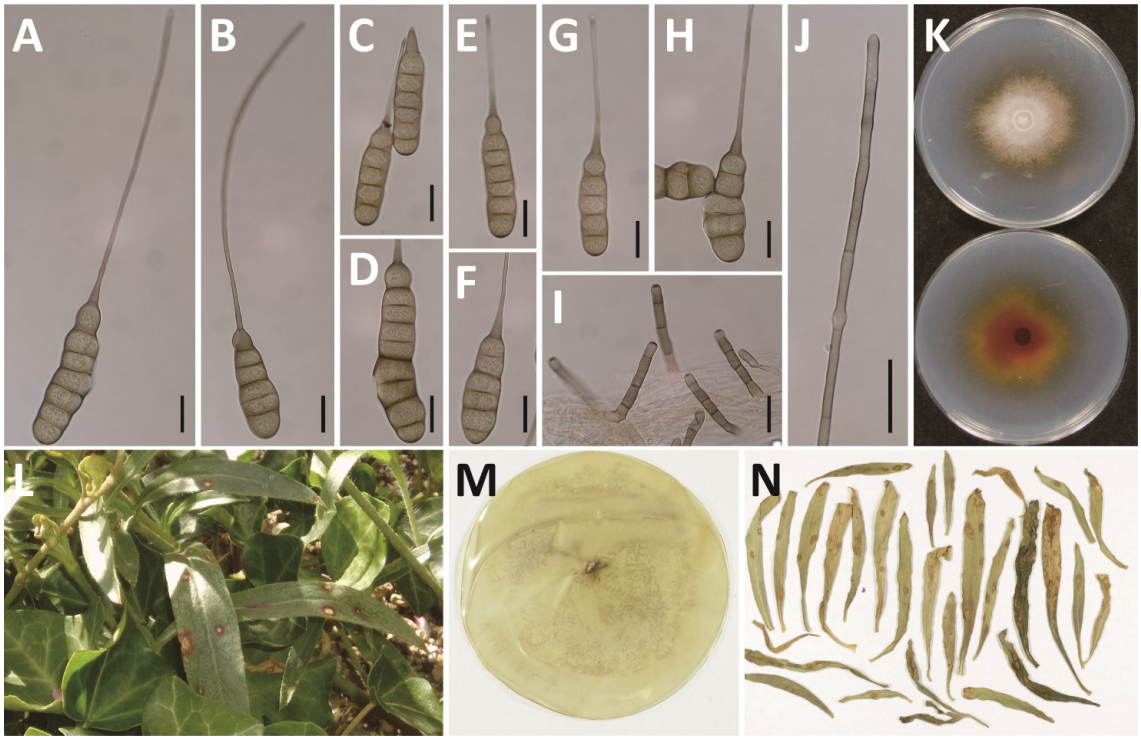

Fig. 5.16. Morphological features of *Alternaria paragomphrenae* (ex-holotype culture MAFF 246768) on V8 juice agar medium. **A–H.** Conidia and lumina. **I.** Conidiophores. **J.** Colored beak. **K.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). **L.** Natural symptoms on *Gomphrena haageana*. **M.** Dried culture specimen ex MAFF 246768 (holotype: TSN-F-85449). **N.** Isotype specimen, MUMH 242310. Bars (A–J) = 25 µm.

Type: **Japan**, Shizuoka Prefecture, Hamamatsu, Hamakita, on leaves of *Gomphrena haageana* Klotzsch, 14 Sep. 2004, *J. Nishikawa*, holotype TNS-F-85449 (a dried culture specimen ex MAFF 246768) [MBT 385038], culture ex-holotype MAFF 246768 = MUCC 1683 = AC7, isotype MUMH 242310.

Host range and distribution: Pathogenic to *Gomphrena* and experimentally to *Alternanthera*, but not to *Celosia* and *Amaranthus* in the inoculation tests conducted (Table 5.4). Its distribution is only known from the type collection.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching an average of 59 ± 2.4 mm in diam after 7 d at 25 °C. They are almost rounded, with inconspicuous margins at the circumference; aerial hypha are cottony, pale gray to white, reverse center pigmented with yellowish to reddish orange; sporulation is sparse.

Teleomorph: Not observed on RSA medium.

Section *Alternaria* D.P. Lawr., Gannibal, Peever & B.M. Pryor, *Mycologia* **105**: 538. 2013. [MB 802304].

Eleven species with three formae speciales of *A. alternata* and one species complex (*A. arborescens*) were recognized by Woudenberg *et al.* (2015). As for *A. alternata*, however, *A. alternata* f. sp. *fragariae* was misassigned (Nishikawa & Nakashima 2019), and *A. viniferae* and *A. capsicicola* seem to be distinct species from this section because of the morphology and phylogeny provided in the original descriptions (Tao *et al.* 2014; Nasehi *et al.* 2014). Four species with two formae speciales and a novel species from Japan are described in the present study.

Alternaria alstroemeriae E.G. Simmons & C.F. Hill, in Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 444. 2007. [MB 505018]. **Figs** 4.1; **5.17**; Fig. 1c–g in Yamagishi *et al.* (2009).

Type: **Australia**, on leaves of *Alstroemeria* sp., Jul. 2005, *C.F. Hill*, holotype BPI 877375 (dried culture ex EGS52.068) [MBT 119829], culture ex-type CBS 118809 = EGS52.068.

Fig. 5.17. Morphological features of Japanese isolates of *Alternaria alstroemeriae* (MAFF 241374) on potato-carrot agar medium. **A–G.** Conidia. **H.** Submerged sporulation in media. **I.** Conidiophores. Bars = 25 µm.

Collection examined: **Japan**, Nagano Prefecture, Matsumoto, on leaves of *Alstroemeria* sp., Jan. 2008, *N. Yamagishi*, living culture MAFF 241374.

Morphological character on PCA medium: Conidia form in short chains of 2–5, with up to 8 conidia, and very occasionally with lateral branches in 5–7 d. Conidiophores are solitary, simple,

or with a few genicula, short and narrow, 3–32 × 3–6 µm. Conidia are ovoid to obclavate, subcylindrical, and brown to dark brown, measuring 7–63 × 5–18 µm in total, with 0–8 transverse septa and 0–3 longitudinal septa, and their surface is often rough; secondary conidiophores (false beaks) are usually short and unbranched, with 1–2 cells. Sporulation occurs in part when submerged in agar substrate; submerged conidia are ovoid to ellipsoid, and are generally smaller than surface conidia, measuring 8–28(–58) × 3–8 µm, with 0–6 transverse septa and no longitudinal septa.

Culture characteristics on PDA medium: Colonies are fast-growing, reaching 74.6 ± 1.6 mm in diam after 7 d at 25 °C, rounded but with an indistinct circumference; aerial hypha are sparse, olive brown to black, reverse center black to dark green; sporulation is abundant on conidiophores arising from aerial and submerged hypha; no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Alstroemeria* (*Alstroemeriaceae*).

Symptoms: Leaf spots are circular to subcircular, 3–12 mm in diam, and are dark brown to black. The same spots also form on stems, followed by defoliation (Yamagishi *et al.* 2009).

Experimental host range: The Japanese isolate shows a restricted host range; it is pathogenic to *Alstroemeria* sp., but not to the other plants formerly classified as *Liliaceae*, including *Lilium*, *Tulipa*, *Allium*, *Asparagus*, and *Hyacinthus* (Table 4.1).

Distribution: Limited in Australia (Simmons 2007), and Japan (Yamagishi *et al.* 2009; Nishikawa & Nakashima 2013).

Distinctive features: Matured and basal conidia are often subcylindrical, rarely with longitudinal septa; conidia appear in short chains (never exceeding 10) in 7 d; sporulation occurs in part when submerged in agar substrate. It is phylogenetically distinguishable from the

other species of this section via its *gapdh*, *tef1*, *rpb2*, *Alt a 1*, and *endoPG* sequences, but not via its ITS and *act* sequences (Fig. 5.3).

Alternaria alternata (Fr.) Keissl., *Beih. Bot. Centralbl., Abt. 2*, **29**: 434. 1912. [MB 119834]. **Fig. 5.18**.

≡ *Alternaria tenuis* Nees, *Syst. Pilze* (*Würzburg*): 72. 1817. [MB 211928].

≡ *Torula alternata* Fr., *Syst. Mycol.* (*Lundae*) **3**: 500. 1832, nom. sanct. [MB 452904].

= *Helminthosporium tenuissimum* Kunze ex Nees & T. Nees, *Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur.* **9**: 242. 1818. [MB 145362].

≡ *Macrosporium tenuissimum* (Nees & T. Nees) Fr., *Syst. Mycol.* **3**: 374. 1832, nom sanct. [MB 238098].

≡ *Clasterosporium tenuissimum* (Nees & T. Nees: Fr.) Sacc., *Syll. Fung.* (*Abellini*) **4**: 393. 1886. [MB 226655].

≡ *Alternaria tenuissima* (Nees & T. Nees: Fr.) Wiltshire, *Trans. Brit. Mycol. Soc.* **18**: 157. 1933. [MB 280005].

= *Macrosporium fasciculatum* Cooke & Ellis, *Grevillea* **6** (37): 6. 1877. [MB 190904].

≡ *Alternaria fasciculata* (Cooke & Ellis) L.R. Jones & Grout, *Bull. Torrey Bot. Club* **24**: 257. 1897. [MB 445678].

= *Macrosporium caudatum* Cooke & Ellis, *Grevillea* **6** (39): 87. 1878. [MB 236061].

≡ *Alternaria caudata* (Cooke & Ellis) E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 496. 2007. [MB 504447].

= *Macrosporium inquinans* Cooke & Ellis, *Grevillea* **7**: 39. 1878. [MB 207381].

= *Macrosporium maydis* Cooke & Ellis, *Grevillea* **6** (39): 87. 1878. [MB 245860].

= *Macrosporium meliloti* Peck, *Annual Rep. New York St. Mus. Nat. Hist.* **33**: 26. 1880. [MB 246007].

= *Macrosporium erumpens* Cooke, Grevillea 12: 32. 1883. [MB 183250]; *Macrosporium erumpens* Cooke, in Ravenel, *Fung. Amer. Exs.*: no. 605. 1882, nom. nud.

≡ *Alternaria erumpens* (Cooke) Joly, *Le Genre* Alternaria: 199. 1964. [MB 326041].

= *Macrosporium martindalei* Ellis & G. Martin, *Amer. Naturalist* **18**: 189. 1884. [MB 246034].

≡ *Alternaria martindalei* (Ellis & G. Martin) Joly, *Le Genre* Alternaria: 209. 1964. [MB 326056].

= *Macrosporium polytrichi* Peck, *Annual Rep. New York St. Mus. Nat. Hist.* **43**: 77. 1890. [MB 243404].

= *Macrosporium podophylli* Ellis & Everh., *Proc. Acad. Nat. Sci. Philadelphia* **43**: 92. 1891. [MB 243735].

≡ *Alternaria podophylli* (Ellis & Everhart) Joly, *Le Genre* Alternaria: 212. 1964. [MB 326067].

= *Macrosporium seguierii* Allesch., *Hedwigia* **33**: 75. 1894. [MB 246499].

= *Macrosporium amaranthi* Peck, *Bull. Torrey Bot. Club* **22**: 493. 1895. [MB 245357].

≡ *Alternaria amaranthi* (Peck) J.M. Hook, *Proc. Indiana Acad. Sci.*: 214. 1921. [MB 250631].

= *Alternaria ribis* Bubák & Ranoj., *Ann. Mycol.* **8** (3): 400. 1910. [MB 203274].

= *Alternaria mali* Roberts, *J. Agric. Res.* **2**: 58. 1914. [MB 214990].

= *Alternaria palandui* Ayyangar, *Bull. Agric. Res. Inst., Pusa* **179**: 14. 1928. [MB 273272].

= *Alternaria lini* Dey, *Indian J. Agric. Sci.* **3** (5): 892. 1933. [MB 269554].

= *Alternaria tenuissima* var. *godetiae* Neerg., *Trans. Brit. Mycol. Soc.* **18**: 157. 1933. [MB 284042].

≡ *Alternaria godetiae* (Neerg.) Neerg., *Aarsberetn. J. E. Ohlens Enkes Plantepatol. Lab.* **10**: 14. 1945. [MB 284029].

= *Macrosporium pruni-mahalebi* Savul. & Sandu, *Hedwigia* **75**: 228. 1935. [MB 275364].

= *Alternaria rumicicola* R.L. Mathur, J.P. Agnihotri & Tyagi, *Curr. Sci.* **31** (7): 297. 1962. [MB 326071].

= *Alternaria angustiovoidea* E.G. Simmons, *Mycotaxon* **25** (1): 198. 1986. [MB 103918].

= *Alternaria pellucida* E.G. Simmons, *Mycotaxon* **37**: 102. 1990. [MB 127808].

= *Alternaria rhadina* E.G. Simmons, *Mycotaxon* **48**: 101. 1993. [MB 360472].

= *Alternaria destruens* E.G. Simmons, *Mycotaxon* **68**: 419. 1998. [MB 444642].

= *Alternaria broussonetiae* T.Y. Zhang, W.Q. Chen & M.X. Gao, *Mycotaxon* **72**: 439. 1999. [MB 460964].

= *Alternaria citriarbusti* E.G. Simmons, *Mycotaxon* **70**: 287. 1999. [MB 460141].

= *Alternaria citrimacularis* E.G. Simmons, *Mycotaxon* **70**: 277. 1999. [MB 460140].

= *Alternaria dumosa* E.G. Simmons, *Mycotaxon* **70**: 310. 1999. [MB 460138].

= *Alternaria interrupta* E.G. Simmons, *Mycotaxon* **70**: 306. 1999. [MB 460137].

= *Alternaria limoniasperae* E.G. Simmons, *Mycotaxon* **70**: 272. 1999. [MB 460136].

= *Alternaria perangusta* E.G. Simmons, *Mycotaxon* **70**: 303. 1999. [MB 461085].

= *Alternaria toxicogenica* E.G. Simmons, *Mycotaxon* **70**: 294. 1999. [MB 461083].

= *Alternaria turkisafria* E.G. Simmons, *Mycotaxon* **70**: 290. 1999. [MB 461082].

= *Alternaria sanguisorbae* M.X. Gao & T.Y. Zhang, *Mycosystema* **19**: 456. 2000. [MB 467719].

= *Alternaria platycodonis* T.Y. Zhang, *Flora Fungorum Sin.* **16**: 66. 2003. [MB 504414].

= *Alternaria yali-inficiens* R.G. Roberts [as '*yaliinficiens*'], *Pl. Dis.* **89** (2): 142. 2005. [MB 356797].

= *Alternaria astragali* Wangeline & E.G. Simmons, *Mycotaxon* **99**: 84. 2007. [MB 510572].

= *Alternaria brassicinae* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 532. 2007. [MB 505029].

= *Alternaria citricancri* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 542. 2007. [MB 505030].

= *Alternaria daucifolii* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 518. 2007. [MB 505026].

= *Alternaria herbiphorbicola* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 608. 2007. [MB 505039].

= *Alternaria postmessia* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 598. 2007. [MB 505036].

= *Alternaria pulvinifungicola* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 514. 2007. [MB 505025].

= *Alternaria seleniiphila* Wangeline & E.G. Simmons, *Mycotaxon* **99**: 86. 2007. [MB 510571].

= *Alternaria soliaegyptiaca* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 506. 2007. [MB 505022].

= *Alternaria tomaticola* E.G. Simmons & Chellemi, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 528. 2007. [MB 505027].

= *Alternaria vaccinii* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 432. 2007. [MB 505016].

Type: Not designated.

Neotype: **L** 910, 262-129 (designated in Simmons 1967).

Epitype: **India**, on *Arachis hypogaea* L., 1 Dec. 1980, *E.G. Simmons*, IMI 254138 (designated in de Hoog & Horré 2002) [MBT 25891], culture ex-epitype CBS 916.96 = ATCC 66981 = EGS34.016.

Collections examined: **Japan**, from seeds of *Vigna radiata* (L.) R. Wilczek, 1998, *T. Sato*, MUMH 11693, living culture MAFF 239887; Nagano Prefecture, Azumino, on leaves of *Impatiens hawkeri* W. Bull, 28 Aug. 2006, *J. Nishikawa*, living culture MUCC 1610 = AC51; Shizuoka Prefecture, Kakegawa, on leaves of *Antirrhinum majus* L., 28 May 2008, *J. Nishikawa*, MUMH 11682, living culture MUCC 1611 = AC54; Kanagawa Prefecture, Nakai, on leaves of *Pelargonium hortorum* Bailey, 29 Sep. 2004, *J. Nishikawa*, MUMH 11672, living culture MUCC 1616 = AC66; Shizuoka Prefecture, Kakegawa, on leaves of *Primula × polyantha*, 6 Nov. 2004, *J. Nishikawa*, MUMH 11674, living culture AC67; Shizuoka Prefecture, Kakegawa, on leaves of *Solanum lycopersicum* L., 28 Jun. 2011, *J. Nishikawa*, living culture AC82; Tokyo, Chiyoda, from seeds of *Vigna radiata*, Dec. 2012, *T. Sato*, living culture MAFF 243775; Kanagawa Prefecture, on leaves of *Pyrus aromatica* Nakai & Kikuchi, 1958, *S. Toyota*, living culture MAFF 305014; Chiba Prefecture, on leaves of *Pyrus aromatica* Nakai & Kikuchi, 1959, *N. Nishihara*, living culture MAFF 305015; on leaves of *Pyrus aromatica*, *M. Kusunoki*, living culture MAFF 410775; Shizuoka Prefecture, Kakegawa, on leaves of *Osteospermum* sp., Jul. 2003, *J. Nishikawa*, living culture AC64; Shizuoka Prefecture, Kakegawa, on leaves of *Eustoma exaltatum* (L.) G. Don subsp. *russellianum* (Hook.) Kartesz, 24 Dec. 2003, *J. Nishikawa*, living culture AC65.

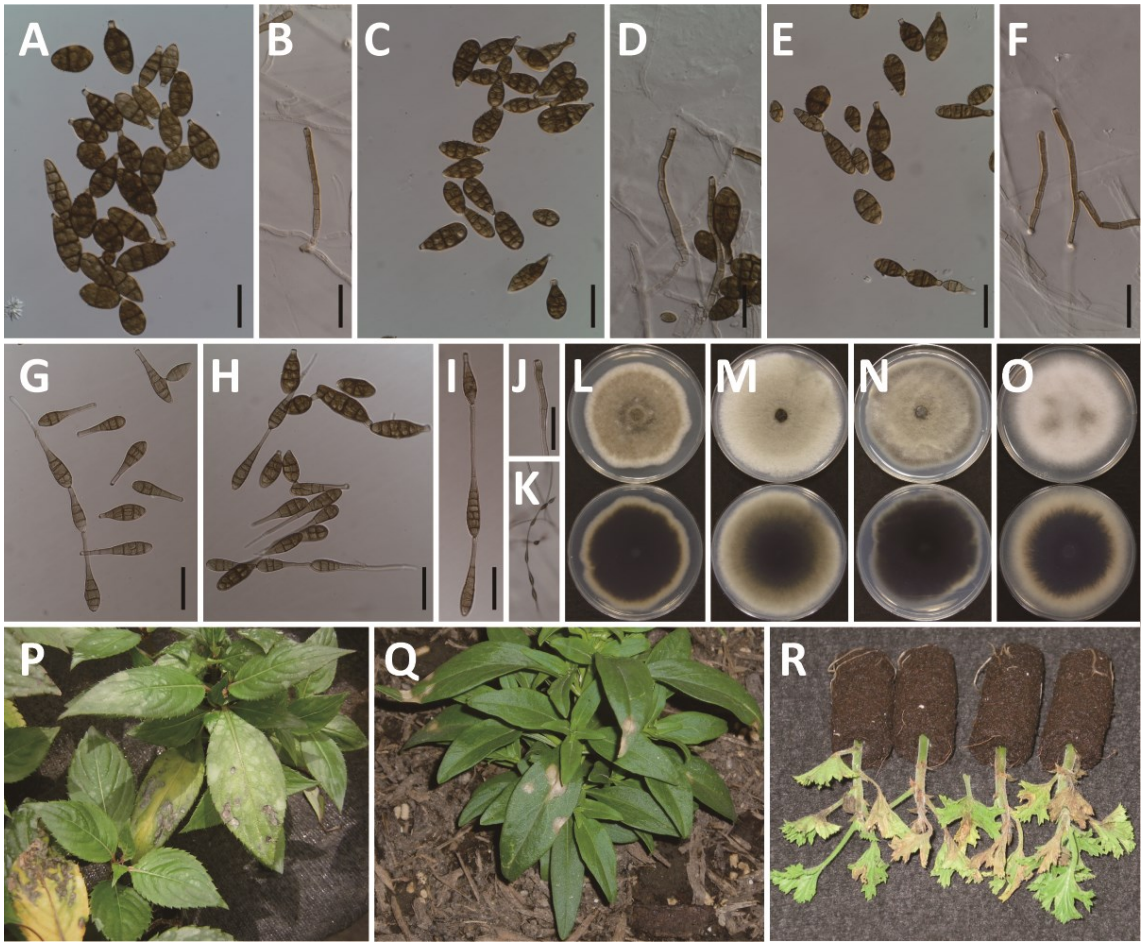

Fig. 5.18. Japanese isolates of *Alternaria alternata*. **A–K.** Conidia and conidiophores on PCA medium ex MUCC 1610 (AC51) (A, B), MUCC 1611 (AC54) (C, D), MUCC 1616 (AC66) (E, F), and MAFF 239887 (E–K). **L–O.** Culture on PDA medium (upper = surface, lower = reverse) of MUCC 1610 (L), MUCC 1611 (M), MUCC 1616 (N), and MAFF 239887 (O). **P–R.** Natural symptoms on *Impatiens hawker* (P), *Antirrhinum majus* (Q), and *Pelargonium hortorum* (R). Bars (A–J) = 25 µm.

Morphological character on PCA medium: Conidia form as complexed, long chains of 10–22, commonly with lateral branches. Conidiophores are solitary, subcylindrical, unbranched, straight or geniculate, and thin, ranging from 15–93 × 3–5 µm, and sometimes proliferate sympodially. Conidia are highly varied, ovoid to ellipsoid, pyriform or obclavate, pale brown to

brown, and are usually smooth; conidial bodies range from 11–50 × 7–18 µm and are 25 × 12 µm on average, commonly not exceeded 50 µm long, with 1–7 transverse and 0–5 longitudinal septa, and they are slightly constricted at the median and some transverse septa. Secondary conidiophores (false beaks) appear at the apical end of conidia, and are short and mostly single-celled, but are often unstable in length (some in isolate MAFF 239887 are elongated, reaching 19–110 µm).

Culture characteristics on PDA medium: Colonies are fast-growing, reaching an average of 77.3 ± 1.3 mm in diam after 7 d at 25°C. They are rounded with white margins at the circumference; aerial hypha are cottony and sometimes sparse, variable in color, pale gray, grayish green to dark green, reverse center black to dark green. Sporulation is abundant; no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: Multiple genera in multiple families serve as hosts, and it is often saprophytic; 692 host records were found in USDA Fungal databases (Farr & Rossman 2018), and 32 diseases in the database of plant diseases in Japan, including three pathotype strains (http://www.gene.affrc.go.jp/databases-micro_pl_diseases_en.php) (Table 1.1).

Symptoms: Necrotic spots on *Impatiens* are sometimes circular and often irregular, measuring 1–8 mm in diam. Leaf spots on *Antirrhinum* are usually fairly circular, measuring 5–10 mm in diam, developing into coalesced lesions that are gray with pale brown margins. Leaf and stem spots on *Pelargonium* seedlings are irregular, then represented as leaf bright. As for isolates MAFF 239887 and MAFF 243775 obtained from *Vigna*, lesions appearing from the roots to the hypocotyls of sprouts appear black and rotten (Sato *et al.* 2014; Sato 2015), and angular to irregular spots are produced on inoculated true leaves (Sato, personal communication).

Distribution: Ubiquitous.

Distinctive features: Small conidia, conidial bodies rarely exceed 50 µm long, and are formed in long chains (mostly over 20 conidia), frequently with lateral branches. Secondary conidiophores are commonly short, consisting of 1–2 cells.

Notes: Woudenberg *et al.* (2015) synonymized 35 names into the species based on their multi-locus phylogeny. Those synonyms include important host selective pathogens, such as *A. mali*, *A. limoniasperae*, and *A. toxicogenica*, which were newly assigned as formae speciales (with pathotypes) of *A. alternata*: *A. alternata* f. sp. *mali*, *A. alternata* f. sp. *citri* pathotype rough lemon, and *A. alternata* f. sp. *citri* pathotype tangerine, respectively. *Alternaria viniferae* Yong Wang bis, Y.Y. Than, K.D. Hyde, X.H. Li had also been synonymized by Woudenberg *et al.* (2015); however, this classification is problematic because of the original description using *gapdh* and *Alt a 1*-based phylogeny, as well as *A. alstroemeriae*-like morphology (Tao *et al.* 2014), which are highly distinguishable from those of *A. alternata*.

Alternaria cylindrica Jun. Nishikawa & C. Nakash., ***sp. nov.*** MycoBank MB 829136. **Figs 5.19**, **20**.

Diagnosis: Long and narrow, cylindrical conidia with few longitudinal septa, which are produced abundantly in long chains and are quite distinctive among members of the genus. Obclavate conidia such as typically seen in sect. *Alternaria* are often produced at the apex and sides of chains. Pathogenicity is selective to *Petunia* among members of the *Solanaceae*. It is phylogenetically close to *A. alternata* and the *A. arborescens* species complex.

Etymology: Named after the Latin “cylindricus”, referring to the shape of the conidia, which are cylindrical.

Descriptions: Leaf spots appear on *Petunia* × *atkinsiana* (*Solanaceae*), which are dark brown to black with a pale brown eye in the center. They are circular to irregular, measuring 4–7 mm in diam, are scattered, and become enlarged and confluent, resulting in blighting of leaves. Conidia grown on V8 medium mostly produces chains of 5–9 conidia (moderately long chain), and lateral branches are frequently present in 5–7 d. Conidia are subhyaline to pale brown, obclavate and cylindrical to long and narrow ovoid, measuring 11–214 µm in total length, with 0–21(–33) transverse and 0–7 (relatively uncommon) longitudinal septa. Conidial bodies measure 11–156 × 5–20 µm with 0–13(–25) transverse and 0–7 (relatively uncommon) longitudinal septa. Their surface is usually smooth, but may very occasionally be faintly rough. Secondary conidiophores (false beaks) are elongated up to 120 × 3–6 µm; conidiophores are narrow and short to moderately long, measuring 28–90 × 4–6 µm. Conidia on lesions typically measure 21–192 µm in total length; conidial bodies measure 11–89 × 6–18 µm with 1–14 transverse and 0–7 longitudinal septa; secondary conidiophores measure up to 121 × 3–6 µm, while conidiophores measure 30–79 × 5–7 µm. Morphology when grown on PCA medium is similar to that observed on V8 medium: 18–270(–340) µm in total length, with 0–30(–43) transverse and 0–4 longitudinal septa; conidial bodies measure 10–115 × 5–14 µm, with 0–19 transverse and 0–4 longitudinal septa; secondary conidiophores measure up to 225 × 3–5 µm, and conidiophores measure 29–98 × 3–6 µm.

Type: **Japan**, Shizuoka Prefecture, Kakegawa, on leaves of *Petunia* × *atkinsiana* (Sweet) W.H. Baxter, 16 Dec. 2006, *J. Nishikawa*, holotype TNS-F-85450 (a dried culture specimen ex MAFF 246770) [MBT 385060], isotype MUMH 11678 and MUMH 11703, culture ex-holotype MAFF 246770 = AC34, GenBank accession number ITS: LC440584.

Host range and distribution: Only known from the type collection examined, but remarkable necrosis lesions with no sporulation were frequently observed on the inoculated leaves of *Solanum lycopersicum* and *S. melongena* during the inoculation tests conducted (Table 5.7).

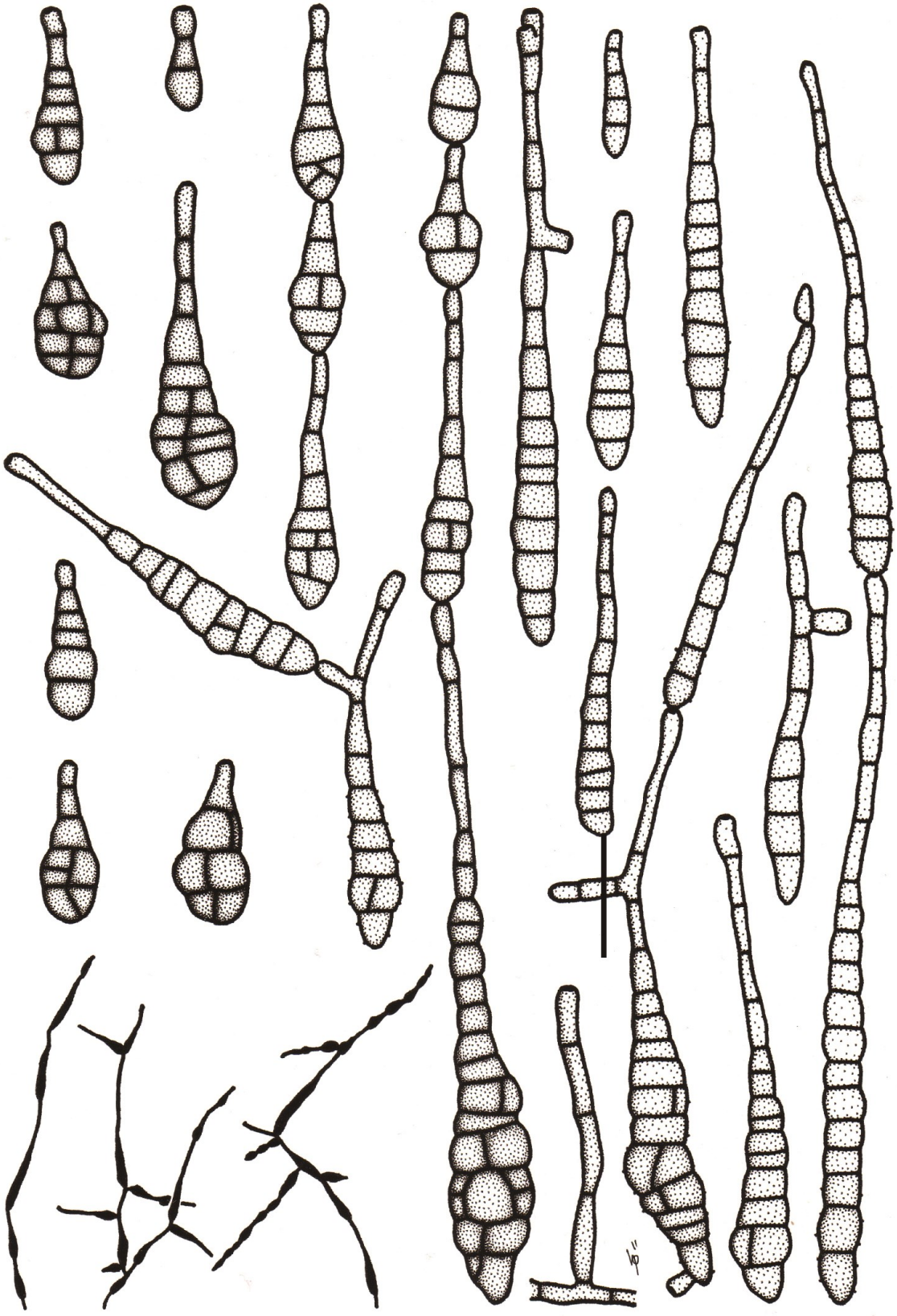

Fig. 5.19. Illustrations of *Alternaria cylindrica* (ex-holotype culture MAFF 246770). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on V8 juice agar medium. Bar = 25 µm.

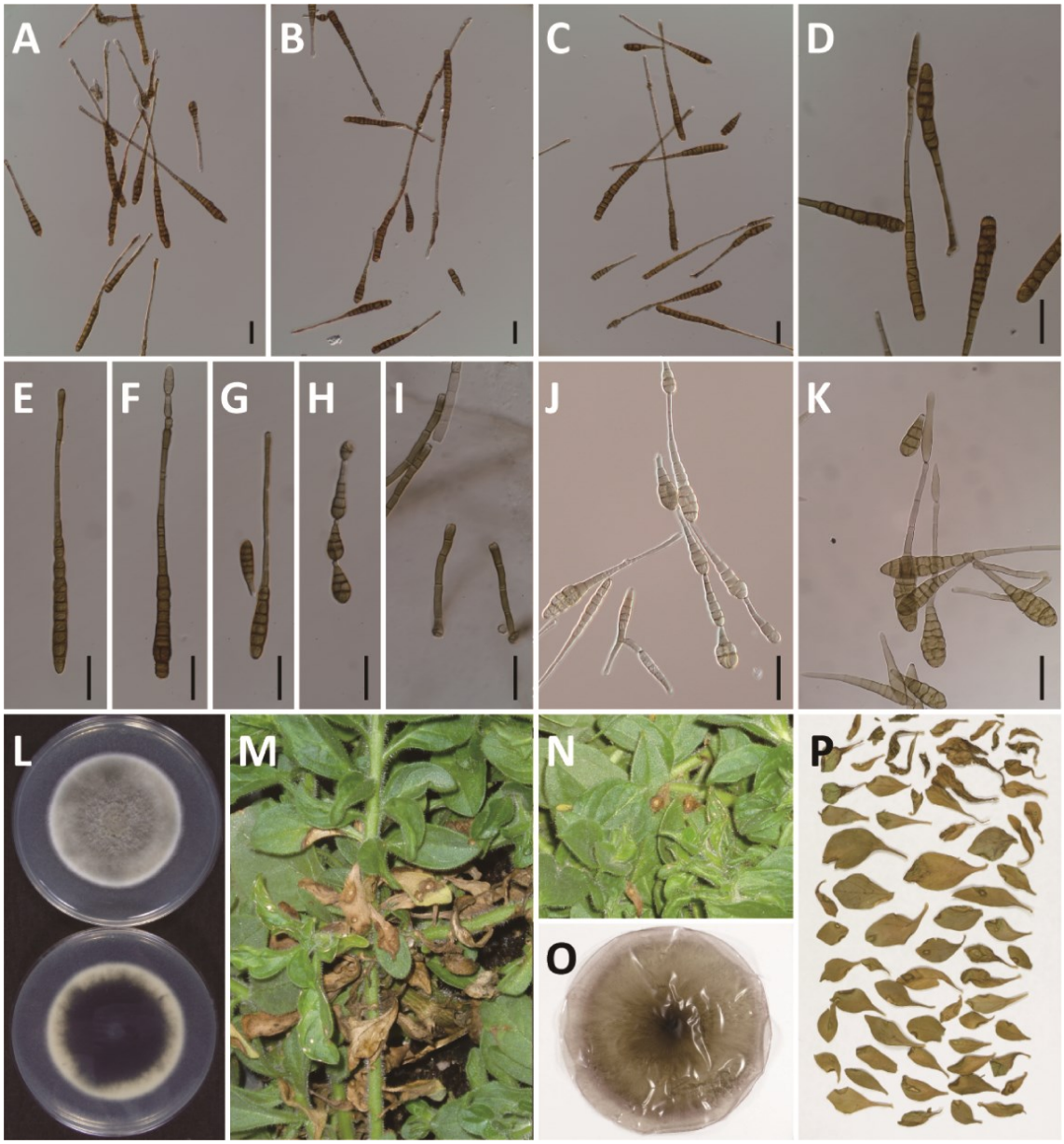

Fig. 5.20. Morphological features of Japanese isolates of *Alternaria cylindrica* (ex-holotype culture MAFF 246770). **A–I.** Conidia and conidiophores on potato-carrot agar medium. **J.** Conidia on V8 juice agar medium. **K.** Conidia on lesion. **L.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). **M–N.** Natural symptoms on *Petunia*. **O.** Dried culture specimen ex MAFF 246770 (holotype: TNS-F-85450). **P.** Isotype specimen. Bars (A–K) = 25 µm.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching 58.4 ± 0.7 mm in diam after 7 d at 25 °C. They are rounded with white margins at the circumference; aerial hypha are cottony, pale gray to grayish or bluish green, reverse center dark green to black, and sporulation is sparse. No pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Notes: Takano (2005) reported a similar disease on petunias, and he identified the pathogen as *Alternaria longissima* Deighton & MacGarvie, which was commonly known as a saprophytic fungus of numerous plants and was characterized as having cercosporoid scolecospore with few longitudinal septa (Deighton & MacGarvie 1968; Ellis 1971). *A. longissima* has since been transferred to the genus *Prathoda* by Simmons (2007), which was supported by molecular phylogenetic analysis indicating that the species should not be treated as an *Alternaria* species (Pryor & Gilbertson 2000). Both our examined isolate and those in Takano (2005) were nearly morphologically identical to each other; however, our examined isolate obviously belongs to a species in the sect. *Alternaria* based on phylogenetic analysis. Although these isolates need to be re-examined, they were probably previously misidentified.

Alternaria gaisen Bokura, *J. Pl. Protect.* (*Tokyo*) **11**: 490. 1924. [MB 823578]. **Figs** 3.1; **5.21**; Fig. 1c–f in Misawa *et al.* (2012).

≡ *Alternaria gaisen* Nagano, *J. Jpn. Hort. Soc.* (*Nihon Engei Zasshi*) **32** (3): 16. 1920, nom. inval. (provisional name; Art. 36.1). [MB 542199].

≡ *Alternaria gaisen* Nagano, in Hara, *Jitsuyo Sakumotsu Byorigaku*: 263. 1925.

≡ *Alternaria gaisen* Nagano ex Hara, *Sakumotsu Byorigaku*, Edn 4: 263. 1928, in Woudenberg *et al*., *Stud. Mycol.* **82**: 15. 2015, nom. superfl. [MB 252306].

= *Alternaria kikuchiana* S. Tanaka, *Mem. Coll. Agric. Kyoto Imp. Univ.* **28** (*Phytopathol. Ser.* **6**): 27. 1933. [MB 268619].

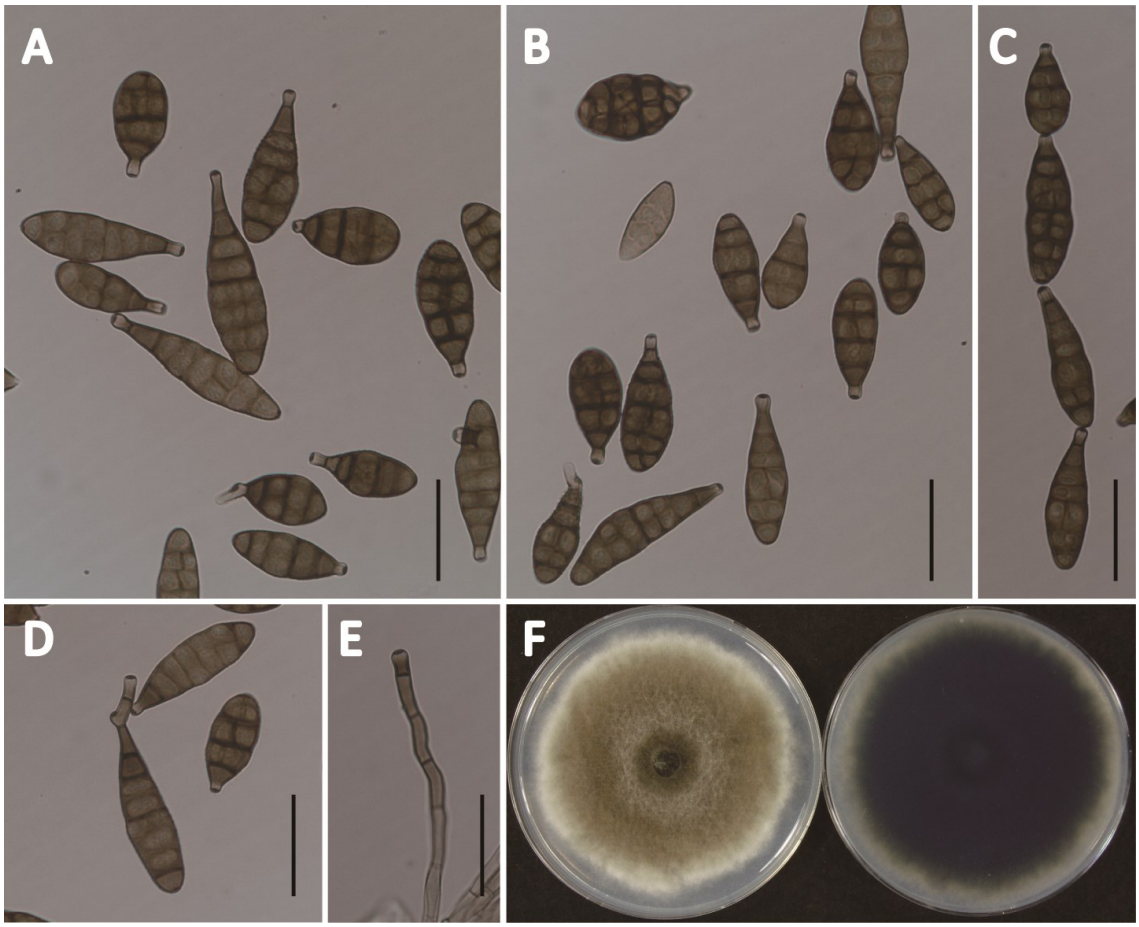

Fig. 5.21. Morphological features of Japanese isolates of *Alternaria gaisen* f. sp. *fragariae* (MAFF 242310) on potato-carrot agar medium. **A–D.** Conidia. **E.** Conidiophore. **F.** Culture on potato-dextrose agar medium (left = surface, right = reverse). Bars = 25 µm.

Type: **Japan**, Nara Prefecture, on leaves of *Pyrus pyrifolia* var. *culta* 'Nijisseiki' (details unknown; not preserved).

Lectotype: Nagano K., *J. Jpn. Hort. Soc.* (*Nihon Engei Zasshi*) **32** (3): 17, Figures (iconotype, selected by Simmons 2007) [MBT 134588].

Epitype: **Japan**, Tottori Prefecture, on *P. pyrifolia* var. *culta*, Jul. 1990, *E.G. Simmons*, dried culture specimen CBS H-22842 (designated in Nishikawa & Nakashima 2019) [MBT 379199],

culture ex-epitype CBS 118488 = EGS90.0391.

Collections examined: **Japan**, Iwate Prefecture, Morioka, on leaves of *Fragaria × ananassa* Rozier 'Morioka-16', 1975, *Y. Watanabe*, living cultures M-11 (MAFF 731001), M-14 (MAFF 731002), M-15 (MAFF 731003), M-17 (MAFF 731004), M-20 (MAFF 731005), M-22 (MAFF 731006), M-23 (MAFF 731007); Tottori Prefecture, Tohaku, Hokuei, Horticultural Research Center, on leaves of *Pyrus pyrifolia* var. *culta* (Makino) Nakai 'Nijisseiki', Jul. 1999, *F. Yasuda*, living cultures 9901A (MUCC 2151), 9903A (MUCC 2152), 9904C (MUCC 2153); Hokkaido, Esashi, on leaves of *Fragaria × ananassa* 'HS-138', Aug. 2007, *T. Misawa*, dried culture specimen MUMH 11698, living culture E-11 (MAFF 242310); Hokkaido, Hokuto, on leaves of *Fragaria × ananassa* 'HS-138', 22 May 2008, *T. Misawa*, MUMH 11681 (inoculated with MAFF 242310).

Morphological character on PCA medium: Conidia form in short chains of 3–13 conidia (usually up to 10), commonly without lateral branches, although branches occasionally arise as short lateral branches from a primary chain. Conidiophores are solitary, subcylindrical, unbranched, straight or geniculate, and thin, measuring 16–86 × 3–5 µm, and proliferating sympodially, with pores for tretic sporulation. Conidia are ovoid to ellipsoid or obclavate, pale to olive brown, smooth to conspicuously verruculous; conidial bodies range from 10–67 × 6–18 µm (27–31 × 11–13 µm on average), though most do not exceeded 50 µm long, with 0–8 transverse and 0–6 longitudinal septa, and they are slightly constricted at the median and some transverse septa. Secondary conidiophores (false beaks) appear at the apical end of conidia and are short, mostly single-celled, and usually unbranched.

Culture characteristics on PDA medium: Colonies are fast-growing, reaching an average of 82.1 ± 0.7 mm in diam after 7 d at 25 °C, and rounded with white margins at circumference. Aerial hypha are cottony, grayish green to dark green, reverse center dark green to black. Sporulation is abundant, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural hosts: *Fragaria* × *ananassa* Duchesne 'Morioka-16', *Pyrus pyrifolia* var. *culta* 'Nijisseiki', and their related cultivars (*Rosaceae*).

Symptoms: Leaf spots on strawberries are circular, 3–12 mm in diam, brown to black with a reddish-brown border and a yellowish halo that is grayish brown at the center, which may be extend by the fungal toxin.

Host range: It is known as a host-selective toxin producer, which is pathogenic to strawberry cultivar 'Morioka-16' and Japanese pear cultivar 'Nijisseiki', and a few of their related lines (Hayashi *et al.* 1992).

Distribution: Limited in Japan (Nagano 1920, Watanabe & Umekawa 1977), New Zealand (Dingley 1970), Korea (Cho & Moon 1980), and Italy (Wada *et al.* 1996).

Distinctive features: Conidia form in short, unbranched chains. It is pathogenic to only a few cultivars of strawberry (cv. Morioka-16) and Japanese pear (cv. Nijisseiki) owing to its production of AF-toxin. Phylogenetically, it is clearly distinguishable from the other species of this section via *Alt a 1* and *endoPG* sequences.

Notes: Within the species, *A*. *gaisen* includes two formae speciales, *A*. *gaisen* f. sp. *pyri* producing the AK-toxin (toxic to Japanese pear), and f. sp. *fragariae* producing the AF-toxin (toxic to strawberry), were recognized (Nishikawa & Nakashima 2019).

Alternaria iridicola (Ellis & Everh.) J.A. Elliott, *Am. J. Bot.* **4**: 450. 1917. [MB 101558]. **Figs 5.22**, **23**.

≡ *Macrosporium iridicolum* Ellis & Everh., *Proc. Acad. Nat. Sci. Philad.* **46** (3): 382. 1894. [MB 207424].

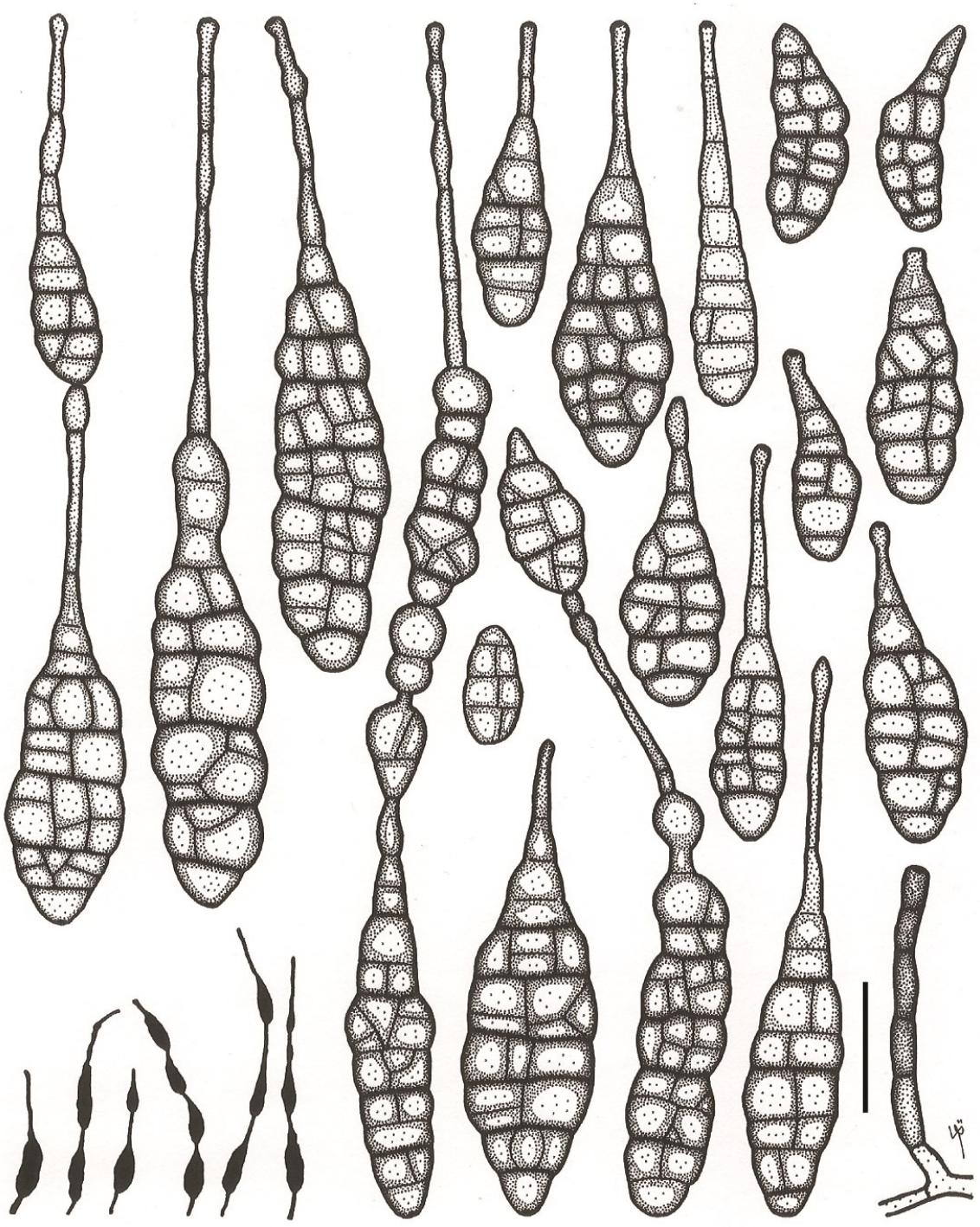

Fig. 5.22. Illustrations of *Alternaria iridicola* (MAFF 246771). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on potato-carrot agar medium. Bar = 25 µm.

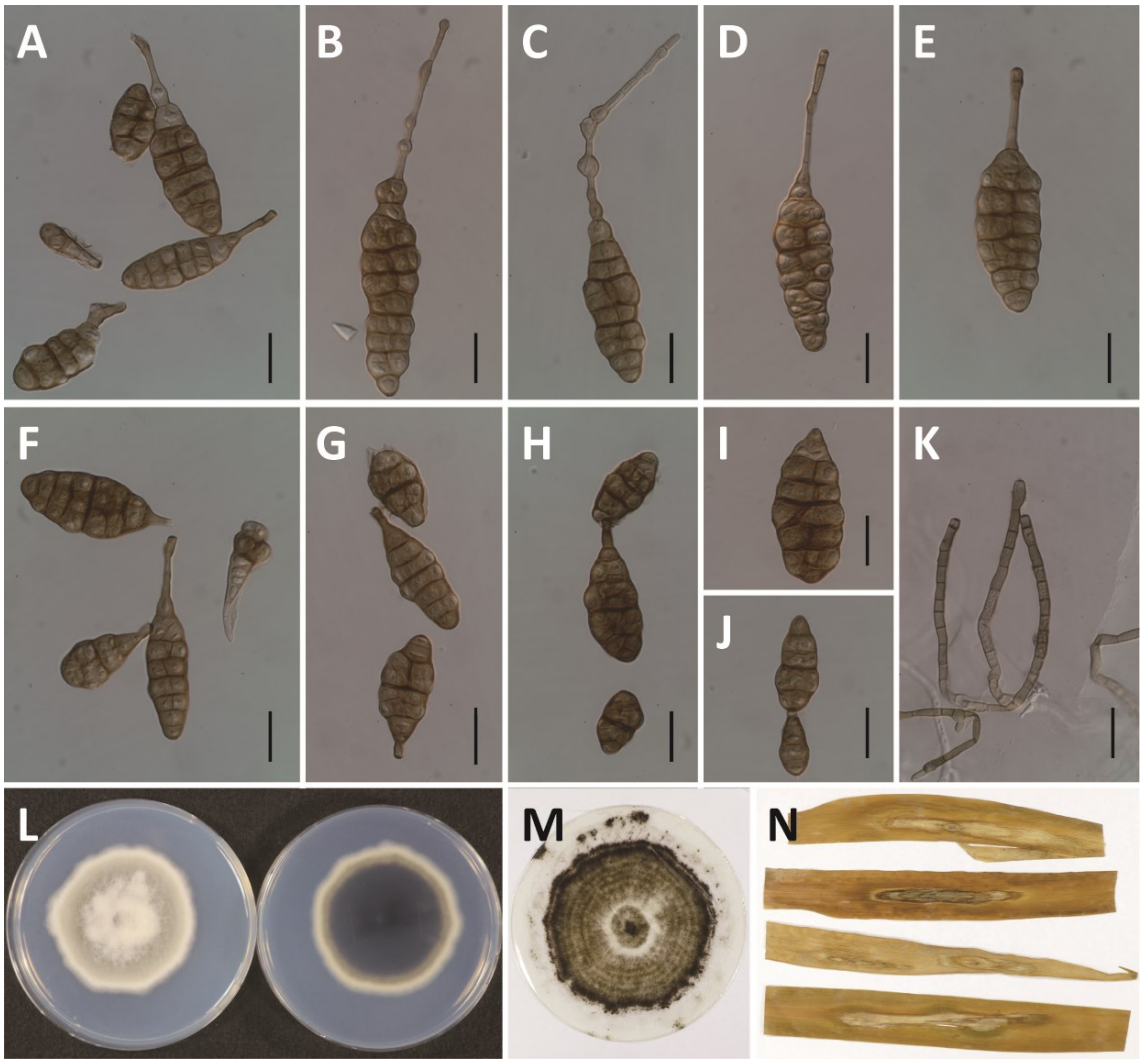

Fig. 5.23. Morphological features of Japanese isolates of *Alternaria iridicola* (MAFF 246890) on potato-carrot agar medium. **A–J.** Conidia. **K.** conidiophores. **L.** Culture on potato-dextrose agar medium; left = surface, right = reverse). **M.** Dried culture specimen ex MAFF 246890 (epitype: TNS-F-85452). **N.** Specimens of diseased leaves of *Iris japonica* (isoepitype: MUMH 11687). Bars (A–E) = 25 µm.

Type: **USA**, Idaho, Moscow, on *Iris missouriensis* Nutt., 27 May 1894, *Henderson*, holotype **NY** 2640 [MBT 121036].

Epitype (**designated here**): **Japan**, Kanagawa Prefecture, Kamakura, on leaves of *Iris japonica* Thunb., 17 Apr. 2013, *H. Horie*, TNS-F-85452 (dried culture specimen of MAFF 246890) [MBT 385027], isoepitypes MUMH 11687and MUMH 11739, culture ex-epitype MAFF 246890 = MUCC 2149 = AC114.

Collections examined: **Japan**, Tokyo, Kodaira, on *Iris japonica* Thunb., 2010, *H. Horie*, living culture MUCC 2148 = AC113; Kanagawa Prefecture, Kamakura, on *I. japonica*, 17 Apr. 2013, *H. Horie*, TNS-F-85452 (**epitype**), MUMH 11687 (**isoepitype**) and MUMH 11739 (**isoepitype**), living culture MAFF 246890 = MUCC 2149 = AC114; Shizuoka Prefecture, Fukuroi, Ugari, on *I. japonica*, 24 Mar. 2018, *J. Nishikawa*, MUMH 11690 and MUMH 11697, living culture MAFF 246771 = MUCC 2501 = AC139.

Morphological character on PCA medium: Conidia are either solitary, or appear in short chains of 3–4 conidia without lateral branches. Conidiophores are solitary to fascicular, subcylindrical, unbranched, straight or sometimes geniculate, and thin, ranging 23–128 × 4–6 µm. Conidia vary in size and appear as distorted, ovoid, ellipsoid to broadly obclavate, or sometimes beakless small oval; they are pale brown to yellowish brown, measuring from 28–311 × 7–38 µm in total. Conidial bodies range from 21–127 × 7–38 µm, with 2–16 transverse and 0–11 longitudinal septa, constricted at some transverse septa, and commonly have a distosepta-like internal wall structure. Secondary conidiophores appear at the apical end of conidia, and are short to long, usually unbranched, often with swollen cells inserted, ranging from 6–200 × 2–7 µm. Conidia of ex-epitype culture MAFF 246890 on PCA medium range from 25–114 × 11–38 µm, with 2–16 transverse and 1–11 longitudinal septa; secondary conidiophores measure 6–200 × 2–7 µm.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching an average of 67.2 ± 3 mm in diam after 7 d at 25 °C, though there are variations among strains, and are almost rounded with white margins at the circumference. Aerial hypha are cottony,

gray to pale grayish-green, reverse center grayish green to dark green. Sporulation is sparse, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Iris* (including *Belamcanda chinensis*) and *Gladiolus* (*Iridaceae*).

Symptoms: Leaf spots on *I. japonica* appear grayish brown surrounded by a yellowish halo. They are distinctly circular, and become enlarged and confluent, with caespituli abundantly formed at the center, and measuring 5–40 mm in diam.

Experimental host range: Restricted to *Iris* spp. excluding *I. ensata*, and non-pathogenic to *Gladiolus* (Table 5.8).

Distribution: Japan, Korea, China, and USA (Shimazaki 1930; Tohyama 1993; Yu 2001; Zhang 2003; Simmons 2007).

Distinctive features: Notably large spores among sect. *Alternaria* commonly arising from narrow conidiophores. Conidia often have a long, secondary conidiophore with cellular swellings. The pathogenicity of this species is restricted to certain *Iris* spp. with some species-selectivity. Phylogenetically, it is clearly distinguishable from the other species of this section in its *gapdh*, *tef1*, *rpb2*, *Alt a 1*, and *endoPG* sequences.

Notes: Based on morphology of the type material, Simmons (2007) suggested that previous morphological descriptions of the species by Elliott (1917), Joly (1964), and Zhang (2003), which described conidial chains and long beaked conidia, could be regarded as those of the other *A. tenuissima*-like species; that is, that they had been misidentified. However, these features were observed on fresh Japanese materials described as the above, and some conidia on epitype material newly designated in the present study also identical with those of type (Simmons 2007). Because diagnostic conidia for the type are scarce, and no reliable living isolates exist in public culture collections, a new epitype was designated in the present study.

Although Yu (2001) has added *Gladiolus* as a natural host in Korea, without details such as photos, no symptoms were observed on the inoculated leaves in this study.

Section *Brassicicola* D.P. Lawr., Gannibal, Peever & B.M. Pryor, *Mycologia* **105**: 541. 2013. [MB 802306].

Five species are recognized in this section based on multi-locus phylogeny reported by Woudenberg *et al.* (2013), and they had excluded *A. japonica* from this section. This section was morphologically characterized by ellipsoidal to ovoid conidia formed in long chains, with apical conidiogenous cells and no or a few longitudinal septa (emending the description of sect. *Brassicicola* sensu by Lawrence *et al.*). However, neither the morphological nor the pathological differences among these species have been defined.

Alternaria brassicicola (Schwein.) Wiltshire, *Mycol. Pap.* **20**: 8. 1947. [MB 292407]. **Figs 5.24**, **25**; Fig. 1 in Nishikawa (2006).

≡ *Helminthosporium brassicicola* Schwein. (as '*brassicola*'), *Trans. Amer. Philos. Soc.* **4** (2): 279. 1832. [MB 149849].

= *Sporidesmium septorioides* Westend., *Bull. Acad. Roy. Sci. Belgique., Cl. Sci., Sér.* 2, **21**: 236. 1854. [MB 141553].

≡ *Alternaria septorioides* (Westend.) E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 570. 2007. [MB 505031].

= *Sporidesmium exitiosum* f. *alternarioides* J.G. Kühn, *Hedwigia* **1**: 91. 1855. [MB 455623].

≡ *Polydesmus exitiosus* f. *alternarioides* (J.G. Kühn) J.G. Kühn, *Hedwigia* **1**: 165. 1858. [MB 455597].

= *Sporidesmium exitiosum* f. *luxuriosum* J.G. Kühn, *Hedwigia* **1**: 91. 1855. [MB 455624].

≡ *Polydesmus exitiosus* f. *luxuriosum* (J.G. Kühn) J.G. Kühn, *Die Krankheiten der Kulturgewächse, ihre Ursachen und Verbreitung*: 165. 1858. [MB 455598].

= *Macrosporium circinans* Berk. & M.A. Curtis, in Curtis, *N. Carol. Geol. Nat. Hist. Surv.* **3**: 128. 1867, nom. nud. [MB 147927].

≡ *Macrosporium cheiranthi* var. *circinans* Berk. & M.A. Curtis, in Berkeley, *Grevillea* **3** (27): 105. 1875. [MB 416457].

≡ *Macrosporium commune* var. *circinans* (Berk. & M.A. Curtis) Sacc., *Syll. Fung.* **4**: 524. 1886. [MB 145451].

≡ *Alternaria circinans* (Berk. & M.A. Curtis) P.C. Bolle, *Meded. Phytopath. Labor. 'WCS'* **7**: 26. 1924. [MB 260901].

= *Alternaria brassicae* var. *minor* Sacc., *Michelia* **2** (6): 172. 1880.

= *Helminthosporium brassicae* Henn., *Hedwigia* **41**: 117. 1902. [MB 150019].

= *Alternaria oleracea* Milbr., *Bot. Gaz.* **74** (3): 321. 1922. [MB 215057].

= *Alternaria brassicae* var. *microspora* J.A. Elliott, in Neergaard, *Danish species of* Alternaria *&* Stemphylium: 129. 1945.

= *Alternaria mimicula* E.G. Simmons, *Mycotaxon* **55**: 129. 1995. [MB 412385].

= *Alternaria solidaccana* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 572. 2007. [MB 505032].

Type: on petioles of *Brassica oleracea* L. var. *capitata* (details unknown; not preserved).

Lectotype: EGS05.167, slide glass preparation "*Helminthosporium brassicola* S [sic] / *valde memoria Beth. in cella nostra*", in the Schweinitz herbarium at **PH** (designated in Simmons 1995a).

Ex-type culture: Unknown.

Collections examined: **Japan**, Shizuoka Prefecture, Kakegawa, on leaves of *Brassica oleracea* L. var. *sabellica* L., 13 Mar. 2003, *J. Nishikawa*, MUMH 11667, living culture MAFF 246772 = AC23; Tokyo, Setagaya, from seeds of *Spinacia oleracea* L., 13 Feb. 2002, *J. Nishikawa*, living culture MAFF 246773 = AC26; Shizuoka Prefecture, Kakegawa, on leaves of *B. oleracea* var. *italica* Plenck, 4 Jun. 2006, *J. Nishikawa*, MUMH 11675, living culture AC27; Shizuoka

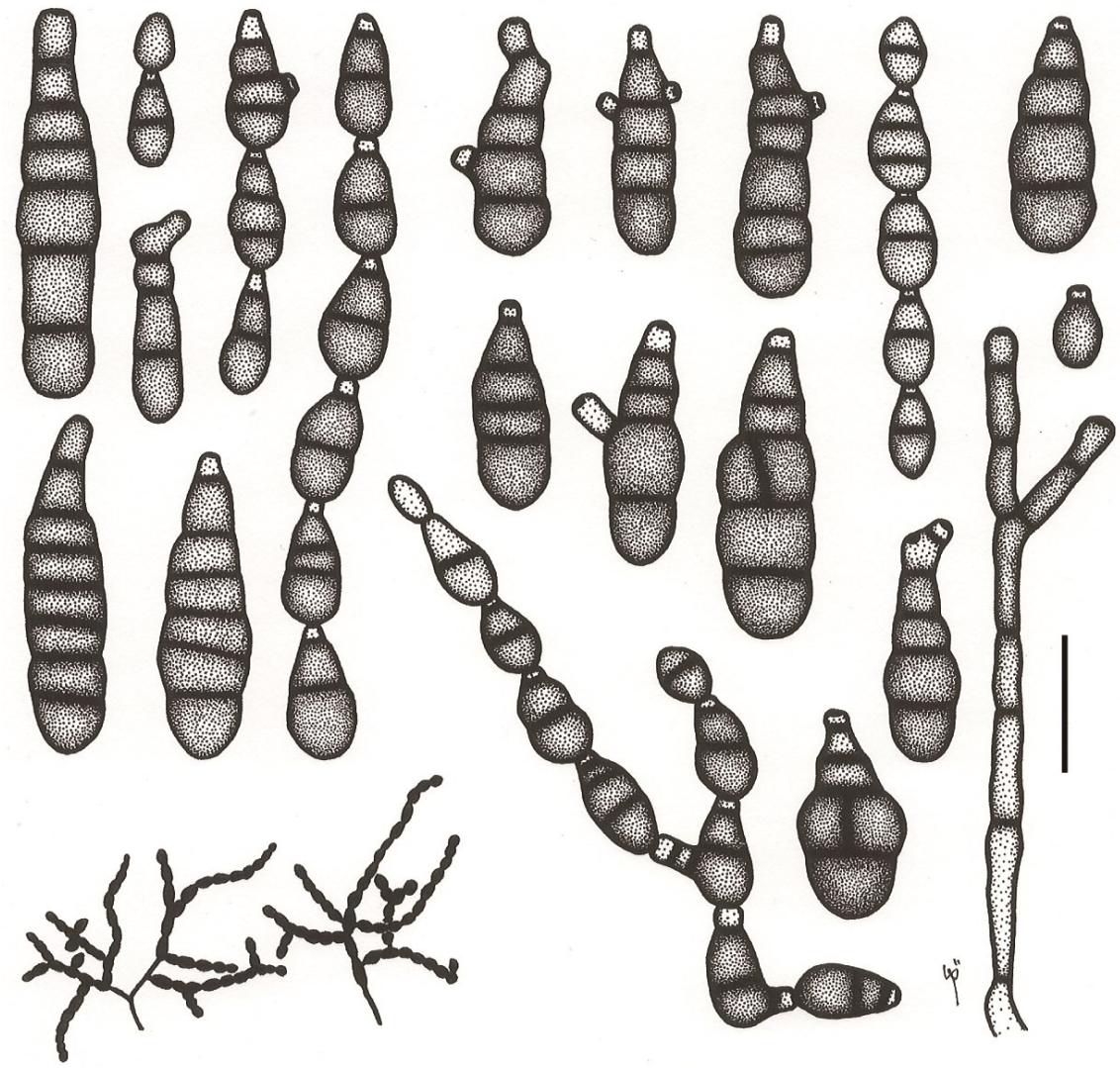

Fig. 5.24. Illustrations of *Alternaria brassicicola* (MAFF 246772). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on potato-carrot agar medium. Bar = 25 µm.

Prefecture, Kakegawa, on leaves of *Brassica rapa* var. *glabra*, 5 Nov. 2008, *J. Nishikawa*, MUMH 11683, living culture MUCC 1612 = AC56; Tokyo, Setagaya, from seeds of *Raphanus sativus* L., Jul. 2000, *J. Nishikawa*, living culture AC70 and AC71; ibid., from seeds of *B. oleracea* var. *italica*, 2001, *J. Nishikawa*, living culture AC72.

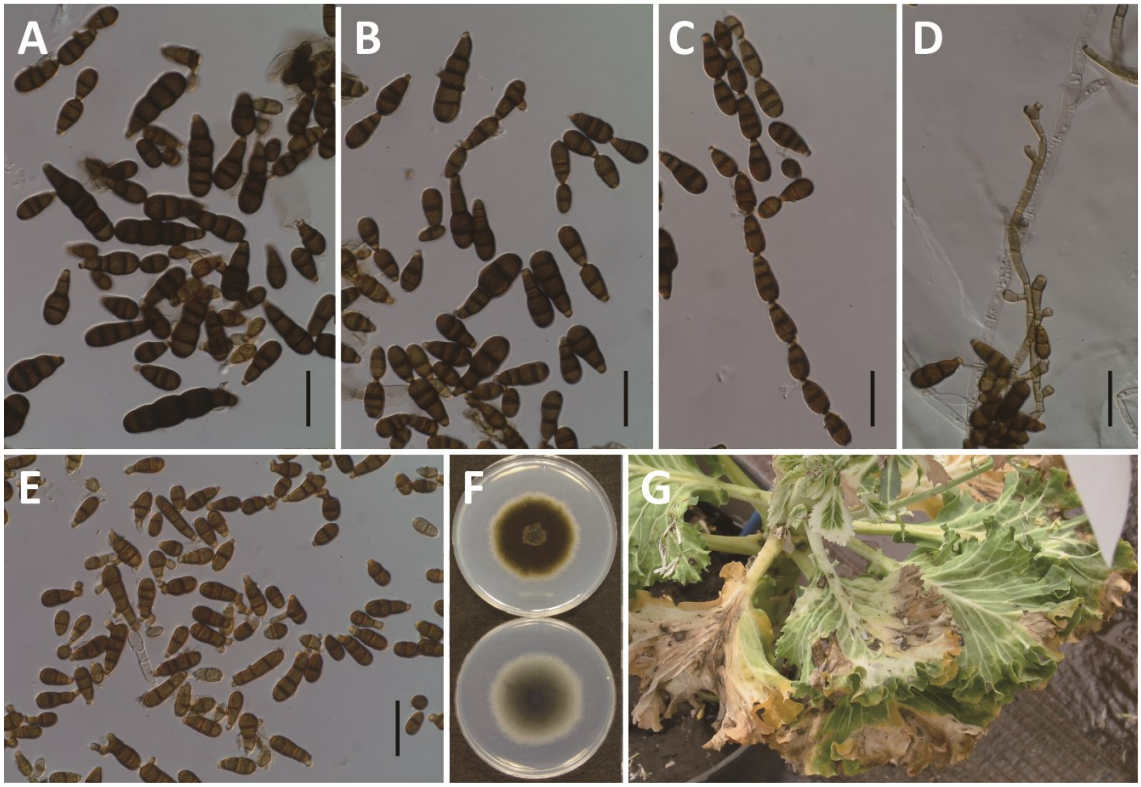

Fig. 5.25. Morphological features of Japanese isolates of *Alternaria brassicicola* on potato-carrot agar medium. **A–D.** Conidia and conidiophores (MAFF 246772). **E.** Conidia ex MAFF 246773. **F.** Culture on potato-dextrose agar medium (MAFF 246772; left = surface, right = reverse). **G.** Natural symptoms on *Brassica oleracea* var. *sabellica*. Bars (A–E) = 25 µm.

Morphological character on PCA medium: Conidia in chains 7–10 more conidia with frequent lateral branches, resulted in 30 more conidial units from one conidiophore. Conidial bodies, ovoid to ellipsoid, subcylindric at mature, brown to dark brown, ranging 8–60 × 6–16 µm, with 0–8 transverse and few longitudinal thicken septa, mostly smooth to occasionally roughened; conidiogenous cell at terminal conidia (secondary conidiophores) short, mostly single-celled. Conidiophores solitary, often branched, straight, intermediately broad, ranging 6–60 × 3–6 µm. Conidia on lesions was also similar but somewhat larger than those on PCA.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching an average of 59.5 ± 1.4 mm in diam after 7 d at 25 °C, and have a rounded circumference. Aerial hypha are sparse, dark green to black, and reverse center gray. Sporulation is abundant, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Brassicaceae*. Simmons (2007) and Farr & Rossman (2018) also recorded on *Digitalis* (*Plantaginaceae*) and non-brassicaceous plants.

Symptoms: Leaf spots on *B. oleracea*, gray to brown, circular to zonate, 8–12 mm in diam, enlarged and confluent; head rot (pin rot) of broccoli and cauliflower, water-soaked to discolored on buds. Caespituli were frequently observed on lesions.

Experimental host range: Strongly pathogenic to *Brassicaceae* including *Diplotaxis* (*Brassiceae*), *Lobularia* (*Alysseae*), *Matthiola* (*Anchonieae*), *Iberis* (*Iberideae*), and *Nasturtium* (*Cardamineae*); weakly pathogenic to *Eutrema* (*Eutremeae*) and *Aubrieta* (*Arabideae*); non-pathogenic to *Capsella* (*Lepidieae*) and non-*Brassicaceae* plants (Table 5.5).

Distribution: Worldwide, including Asia (Japan, Korea, China, etc.), Europe (France, Russia, Denmark, etc.), North and Latin America (USA, Brazil, Canada, etc.), Africa (Ghana, Sudan, South Africa, etc.), and the Pacific (Australia, Cook, New Zealand, etc.) (Yoshii 1941; Ellis 1971; Yu 2001; Zhang 2003; Farr & Rossman 2018).

Distinctive features: Small conidia form in long chains, frequently with lateral branches and rarely with longitudinal septa. Basal conidia form in subcylindrical to oblong. This species was widely pathogenic to *Brassicaceae*, but non- or weakly to *Eutrema*, *Aubrieta* and *Capsella*. It is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Notes: *Alternaria septorioides*, *A. mimicula*, and *A. solidaccana* were synonymized in the present study based on their phylogeny using each ex-type isolate, morphological similarity,

restricted host range within brassicaceous plants, and the ubiquitousness and saprophytic habit of the species. Likewise, *A*. *conoidea* is a possible synonym of the species, and, therefore, sect. *Brassicicola* may be a monotypic lineage.

Section *Crivellia* (Shoemaker & Inderb.) Woudenb. & Crous, *Stud. Mycol.* **75**: 189. 2013. [MB 803735].

≡ *Crivellia* Shoemaker & Inderb., *Canad. J. Bot.* **84**: 1308. 2006. [MB 522560].

Two species are assigned in this section, which is morphologically characterized by cylindrical conidia forming in chains of geniculate conidiophores and microsclerotia formation (Woudenberg *et al.* 2013). Both of the species are known as pathogenic to *Papaver* spp., having a sexual morph formerly known as *Crivellia* (Inderbitzin *et al.* 2006).

Alternaria penicillata (Corda) Woudenb. & Crous, *Stud. Mycol.* **75**: 190. 2013. [MB 803692]. **Fig. 5.26**.

≡ *Brachycladium penicillatum* Corda, *Icon. Fung.* **2**: 14. 1838. [MB 222946].

≡ *Dendryphion penicillatum* (Corda) Fr., *Summa Veg. Scand., Sect. Post.* (*Stockholm*): 504. 1849. [MB 186291].

≡ *Dendryphion penicillatum* (Corda) Fr. var. *penicillatum*., *Summa veg. Scand., Sectio Post.* (*Stockholm*): 504. 1849. [MB 426075].

= *Cucurbitaria papaveracea* De Not., *Sferiacei Italici*: 62. 1863. [MB 245489].

≡ *Pleospora papaveracea* (De Not.) Sacc., *Syll. Fung.* **2**: 243. 1883. [MB 166582].

≡ *Crivellia papaveracea* (De Not.) Shoemaker & Inderb., *Canad. J. Bot.* **84**: 1308. 2006. [MB 245489].

= *Dendryphion penicillatum* var. *sclerotiale* M.-E. Meffert, *Z. ParasitKde* **14** (5): 462. 1950, nom. nud. (Art. 36). [MB 346719].

Type: **Czech**, Praha, on *Papaver* sp., 6 Dec. 1837, DAOM 49356 in **PR**.

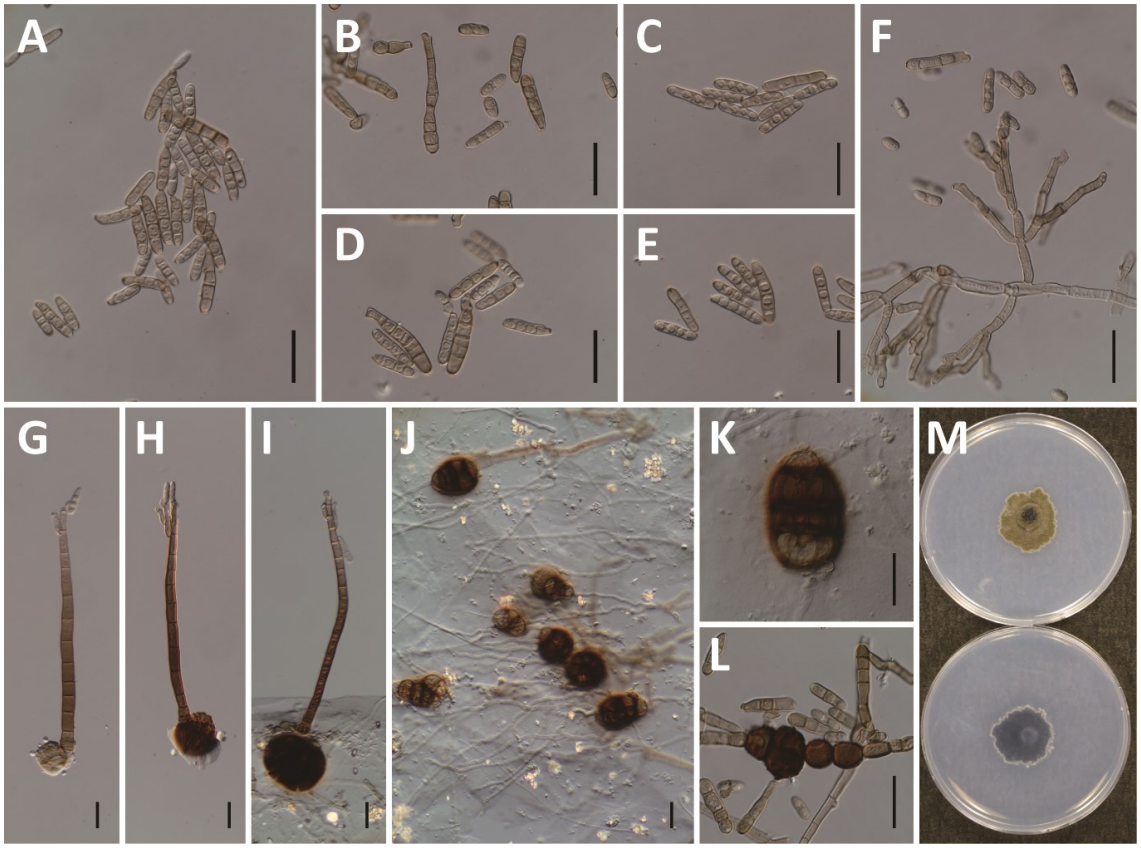

Fig. 5.26. Morphological features of Japanese isolates of *Alternaria penicillata* (AC102). **A–F.** Conidia and microconidiophores on V8 juice agar (V8) medium. **G–L.** Macroconidiophores and microsclerotia on V8. **M.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). Bars (A–L) = 25 µm.

Epitype: **Austria**, Vienna, on stems of *Papaver rhoeas* L., DAOM 230456 (P354) (designated in Inderbitzin *et al.* 2006), culture ex-epitype P354.8 = CBS 116608.

Collection examined: **Japan**, Tokyo, Tachikawa, on leaves of *Papaver nudicaule* L., 13 Jun. 2005, *Y. Makizumi*, living culture MUCC 1657 = AC102.

Morphological character on V8 medium: Conidia commonly appear in short chains of 2–5. They are ellipsoid to cylindrical, subhyaline to pale brown, and measure 8–38 × 4–6 µm in total,

with 0–6 transverse and no longitudinal septa, remaining as distosepta-like, smooth structures. Conidiophores arise from aerial mycelia (microconidiophores), are geniculate with sympodial proliferation, and branches are often arboroid, short and narrow, measuring 19–98 × 3–6 µm. Conidiophores arise from globose knotted cells (Macroconidiophores), are brown to reddish brown, straight and long, and bear short, sub-hyaline conidiophores at the apex measuring 128–225 × 8–11 µm. Cells appear globose and knotted, are brown to dark reddish brown, and measure 30–65 × 25–45 µm. Intercalary microsclerotia (chlamydospores) are dark brown and roughened, appear in knots or chains, and measure 11–29 × 10–24 µm.

Culture characteristics on PDA medium: Colonies are slow-growing, reaching 28.7 ± 4.5 mm in diam after 7 d at 25 °C, and are irregular with white margins at the circumference. Aerial hypha are cottony, olive brown, reverse center dark green to black. Sporulation is abundant, and no pigment is released into the medium.

Teleomorph: Known formerly as the genus *Crivellia*, but not observed on RSA in the present study.

Natural host: *Papaver* (*Papaveraceae*).

Distribution: Worldwide, including Asia (Japan, Korea, India, etc.), Europe (Austria, Turkey, Russia, etc.), North and Latin America (USA, Colombia, and Venezuela), and South Africa (Hirayama & Imura 1941; Richardson 1990; Farr *et al.* 2000; Inderbitzin *et al.* 2006; Hyun *et al.* 2012; Gasich *et al.* 2013; Woudenberg *et al.* 2013; Farr & Rossman 2018).

Distinctive features: Subhyaline to pale brown conidia appearing in chains, and macroconidiophores, microconidiophores, and microsclerotia are also present. It is phylogenetically distinguishable via its ITS sequence (Fig. 5.3).

Section *Eureka* Woudenb. & Crous, *Stud. Mycol.* **75**: 193. 2013. [MB 803739].

Six species are assigned to this section, which are characterized by simple, short, and broad conidiophores, and ellipsoidal to cylindrical conidia that are either solitary or appear in short chains (Woudenberg *et al.* 2013). As to *A*. *eureka*, the type species of this section, a sexual morph has been reported (Simmons 1986).

Alternaria cumini E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 664. 2007. [MB 504417]. **Figs 5.27**, **28**.

Type: **India**, Gujarat, Karli, on *Cuminum cyminum* L., Jan. 1954, *M.K. Patel*, holotype BPI 877406 (dried culture specimen ex CBS 121329) [MBT 119858], culture ex-holotype CBS 121329 = EGS04.1581.

Collections examined: **Japan**, Shizuoka Prefecture, Kakegawa, on leaves of *Cuminum cyminum* L., 17 May 2012, *J. Nishikawa*, living culture MAFF 246774 = AC94; ibid., 18 May 2013, *J. Nishikawa*, living culture AC115.

Morphological character on V8 medium: Conidia are solitary, rarely in chains, brown to dark brown, obclavate to long ellipsoid, subcylindrical, smooth, and measure 23–76 × 8–26 µm, with 1–9 transverse and 0–5 longitudinal septa, slightly constricted at each transverse segment. They are beakless, but most have a conical cell at the apex. Conidiophores are erect, short, and narrow, measuring 10–60 × 5–7 µm. Morphology observed when grown on PCA medium is similar to that observed on V8 medium.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching an average of 62.7 ± 1.1 mm in diam after 7 d at 25 °C. They are rounded with white to pale gray margins at the circumference. Aerial hypha are cottony, pale gray to grayish green, and reverse center brownish green to dark green. Sporulation is sparse, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

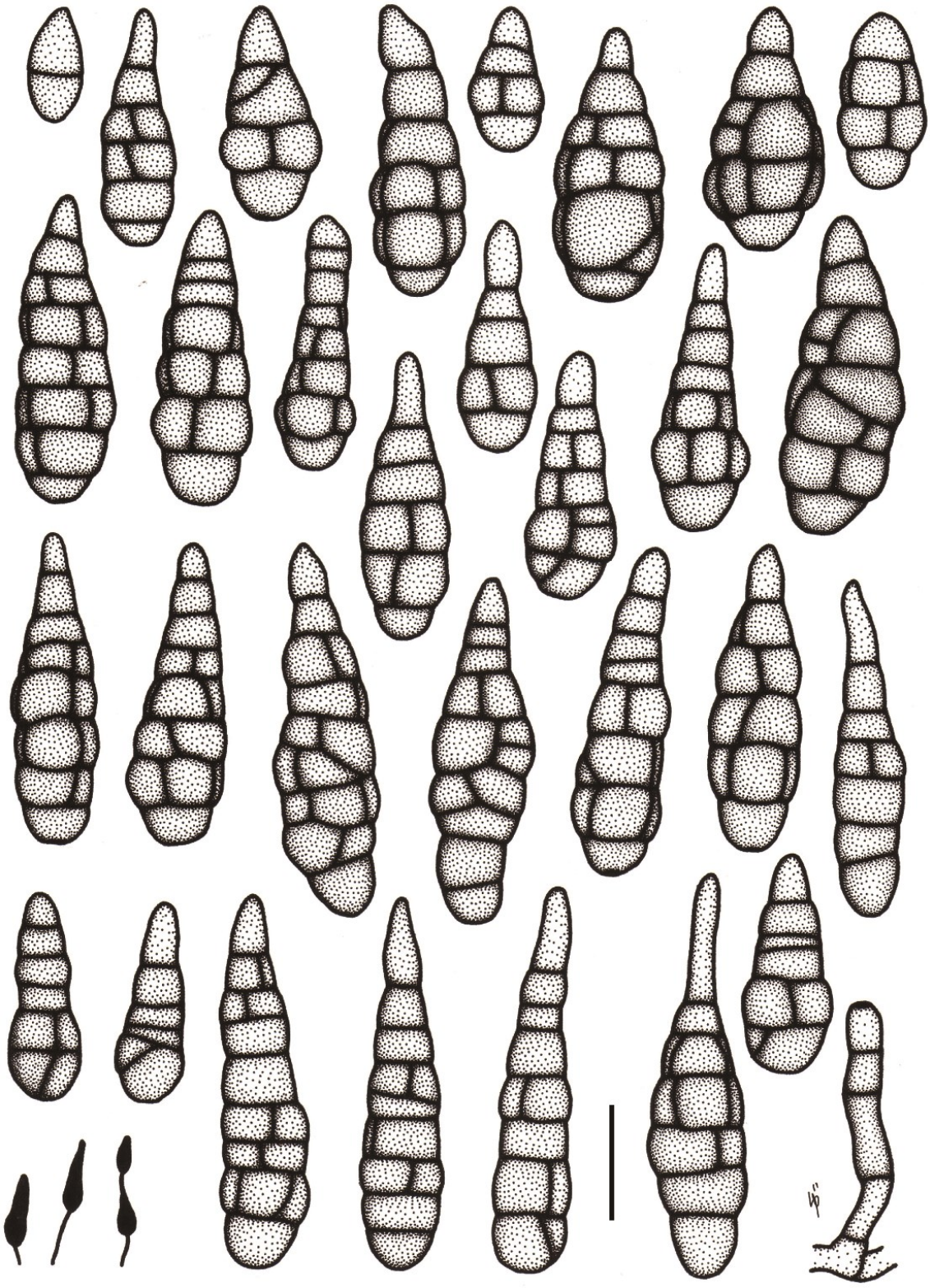

Fig. 5.27. Illustrations of *Alternaria cumini* (MAFF 246774). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on V8 juice agar medium. Bar = 25 µm.

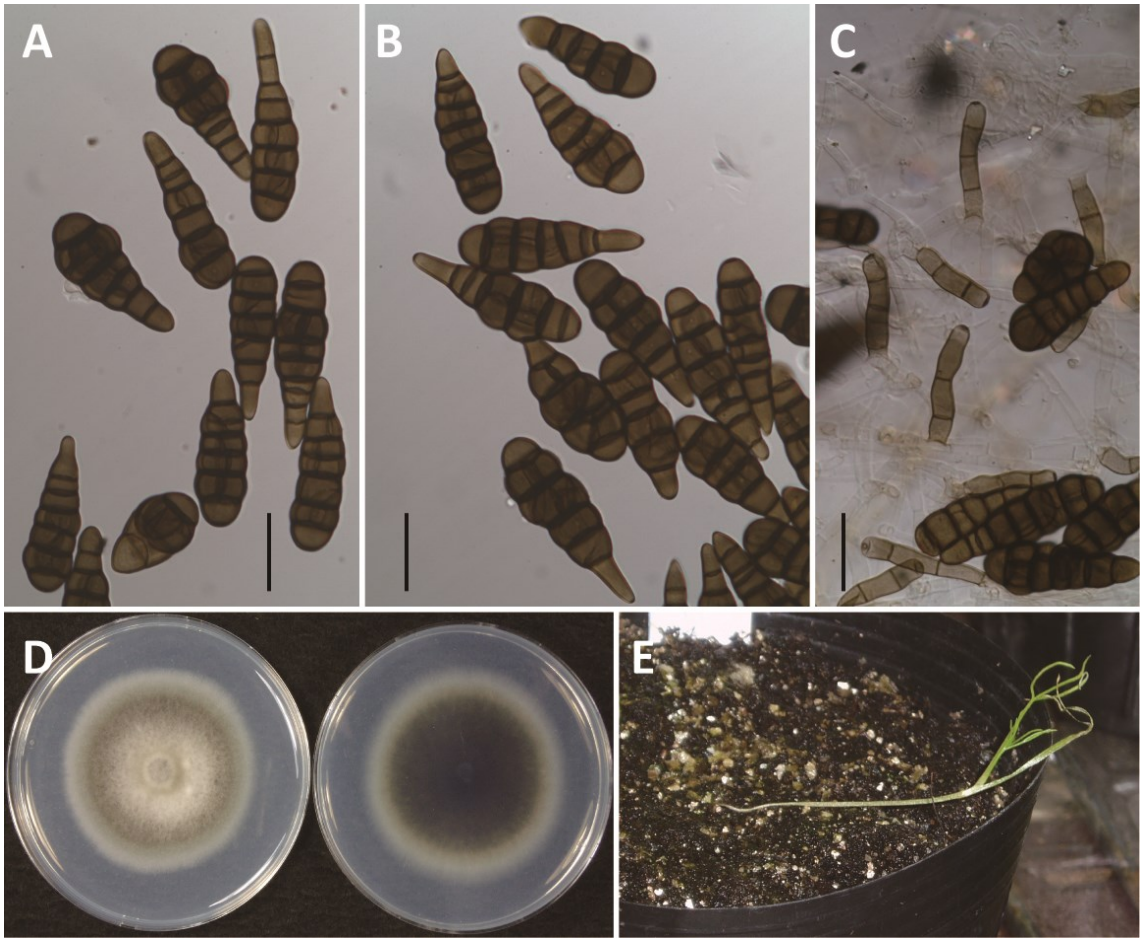

Fig. 5.28. Morphological features of Japanese isolates of *Alternaria cumini* (MAFF 246774). **A–C.** Conidia and conidiophores on V8 juice agar medium. **D.** Culture on potato-dextrose agar medium (left = surface, right = reverse). **E.** Natural symptoms (damping-off of seedlings and leaf blight) on seedling of *Cuminum cyminum*. Bars (A–C) = 25 µm.

Natural host: *Cuminum* (*Apiaceae*).

Symptoms: Damping-off of *Cuminum* seedlings, and experimentally-caused leaf blight.

Experimental host range: Strongly pathogenicity to *Cuminum*, but not to other *Apiaceae* plants, including *Daucus* (Table 5.6).

Distribution: Japan and India (Simmons 2007).

Distinctive features: Beakless, solitary conidia with a conical apical cell. Conidiophores are long and broad. This species shows limited pathogenicity to *Cuminum*, and it is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Notes: This is the first record other than type locality.

Section *Gypsophilae* D.P. Lawr., Gannibal, Peever & B.M. Pryor, *Mycologia* **105**: 541. 2013. [MB 802307].

There are eight species assigned in this section (Woudenberg *et al.* 2013). This section is morphologically characterized by large conidia formed in short chains, with a short, blunt-tapered false beak and multiple septa. All the recognized species in this section were sourced from caryophyllaceous; however, their morphological and pathological differences are not resolved.

Alternaria nobilis (Vize) E.G. Simmons, *Mycotaxon* **82**: 7. 2002. [MB 374014]. **Fig. 5.29**.

≡ *Macrosporium nobile* Vize, in Cooke, *Grevillea* **5** (35): 119. 1877. [MB 241156].

= *Alternaria dianthi* F. Stevens & J.G. Hall, *Bot. Gaz.* **47** (5): 413. 1909. [MB 210395].

≡ *Macrosporium dianthi* F. Stevens & J.G. Hall, in Bewley, *Diseases of Glasshouse Plants*: 106. 1923, nom. inval. (Art. 53.1). [MB 439598]. non *Alternaria dianthi* J.V. Almeida & Sousa da Câmara, *Revista Agron.* **1**: 59. 1903. [MB 505061].

Type: **UK**, Forden, on stems and leaves of *Dianthus caryophyllus* L., 1877, *J.E. Vize*. (holotype not specified).

Lectotype: **K**, EGS11.014 (designated in Simmons 2002), isolectotype (probable) IMI 57062 (*J. E. Vize*, Micro-Fungi Britannici no. 63, *Macrosporium nobile* Vize 1878).

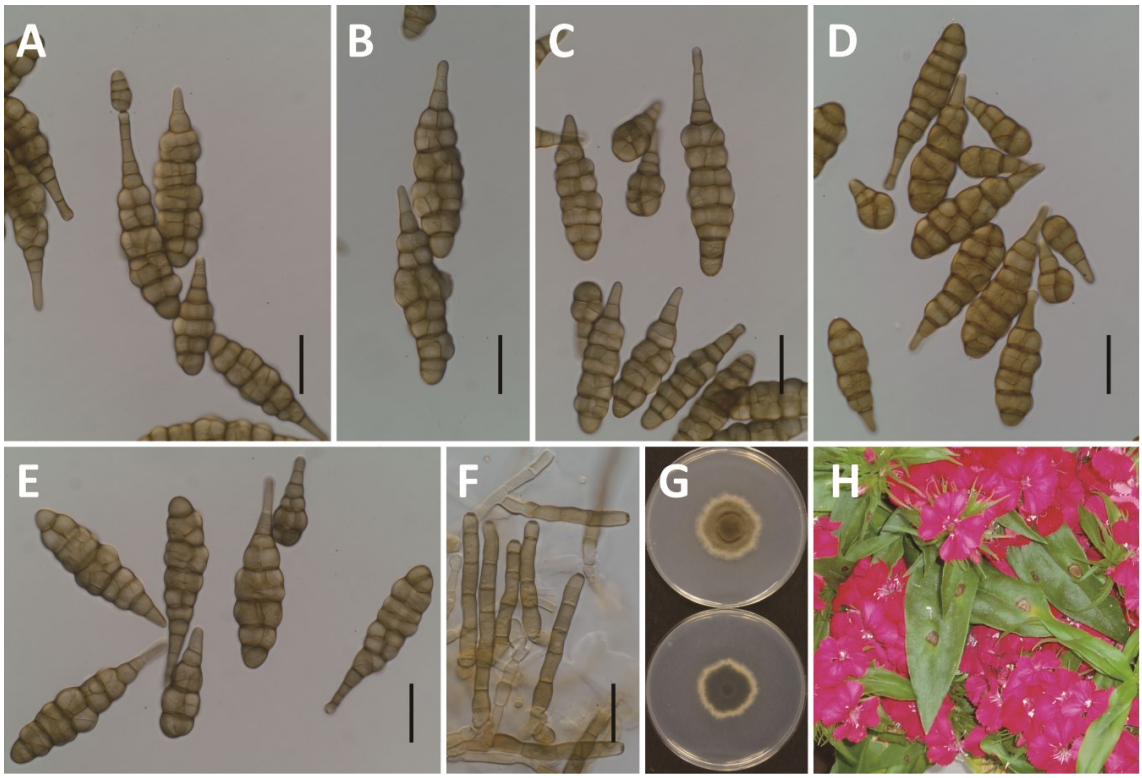

Fig. 5.29. Morphological features of Japanese isolates of *Alternaria nobilis* (AC1) on V8 juice agar medium. **A–E.** Conidia. **F.** Conidiophores. **G.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). **H.** Natural symptoms on *Dianthus barbatus*. Bars (A–F) = 25 µm.

Ex-type culture: Unknown.

Collections examined: **Japan**, Shizuoka Prefecture, Kakegawa, on leaves of *Dianthus barbatus* L., 5 Jun. 2003, *J. Nishikawa*, living culture AC1; Miyagi Prefecture, Sendai, on leaves of *Dianthus caryophyllus* L., 12 Nov. 2002, *Y. Makizumi*, living culture AC25.

Morphological character on V8 medium: Conidia may be solitary, but they commonly appear in chains of 2–5 conidia, without or rarely with lateral branches. They are yellowish

brown to brown, oblong to long obclavate, with a blunt-tapered false beak, almost straight, not swollen, smooth, and are clearly constricted at each transverse septa, ranging from 14–141 µm in total length. Conidial bodies range from 14–100 × 6–30 µm, with up to 16 transverse septa and 13 longitudinal septa. False beaks are not filamentous, usually unbranched and short, consist of 2–3 cells, and ranging from 3–52 × 3–6 µm. Conidiophores are erect, broad, pale brown to brown, and unbranched, ranging from 16–60 × 5–9 µm.

Culture characteristics on PDA medium: Colonies are slow-growing, reaching an average of 39.2 ± 1.4 mm in diam after 7 d at 25 °C. They are rounded to irregular, with white margins at the circumference. Aerial hypha are cottony and dense, grayish green, and reverse center dark green to black. Sporulation is sparse, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: Primarily *Dianthus*, *Silene*, and *Gypsophila* (*Caryophyllaceae*), and also recorded on *Calendula* (*Asteraceae*), *Hibiscus* (*Malvaceae*), *Jasminum* (*Oleaceae*), and *Sesamum* (*Pedaliaceae*).

Symptom: Leaf spots on *D. barbatus* are circular to zonate, grayish brown to brown, and become enlarged and confluent, reaching 4–7 mm with a necrotic eye at the center, and sometimes with a chlorotic halo around the primary lesion.

Distribution: Worldwide, including Asia (Japan, Korea, India, etc.), Europe (Italy, UK, Russia, etc.), North and Latin America (USA, Puerto Rico, Brazil, etc.), Africa (South Africa, Morocco, Tanzania, etc.), and the Pacific (New Zealand, and Australia) (Imai 1914; Rao 1969; Ellis 1971; Richardson 1990; Cho *et al.* 2001; Yu 2001; Garibaldi *et al.* 2013; Woudenberg *et al.* 2013; Farr & Rossman 2018).

Distinctive features: Large conidia, solitary to in short chain with a blunt-tapered false beak and multiple septa. Conidiophores long and broad. This species is pathogenic to *Dianthus*.

Notes: Morphological characteristics of the examined Japanese isolates were well identical to those of *A*. *nobilis* described in Simmons (2002). However, that was also quite similar to those of the other members in sect. *Gypsophilae*, and the results of phylogenetic analysis in this study suggested that *A*. *ellipsoidea* and *A*. *saponariae* were conspecific to the species (Figs 5.2, 5.3).

Section *Japonicae* Woudenb. & Crous, *Stud. Mycol.* **75**: 197. 2013. [MB 803741].

Woudenberg *et al.* (2013) assigned two species to this section, which is morphologically characterized by short- to long-ovoidal conidia formed in short chains, with constrictions at the septa. However, neither the morphological nor the pathological differences between these two species have been well defined.

Alternaria japonica Yoshii, *J. Pl. Protect.* (*Tokyo*) **28**: 17. 1941. [MB 284033]. **Figs 5.30**, **31**.

≡ *Alternaria brassicae* (Berk.) Sacc. var. *macrospora* [non Sacc.] sensu Yoshii, *Bult. Sci. Fak. Terk. Kjusu Imp. Univ.* **5** (3): 224. 1933.

= *Alternaria raphani* J.W. Groves & Skolko, *Canad. J. Res., Sect. C* **22** (5): 227. 1944. [MB 284035].

= *Alternaria matthiolae* Neerg., *Danish species of* Alternaria *and* Stemphylium: 184. 1945. [MB 282388].

= *Alternaria nepalensis* E.G. Simmons, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 480. 2007. [MB 505020].

Type: **Japan**, on leaves of *Brassica rapa* L. and *Raphanus sativus* L. (details unknown, not specified, unpreserved) [MBT 127697].

Lectotype: IMI 876 (designated in Tohyama & Tsuda 1990; the same specimen was designated as a neotype in Simmons 1995a).

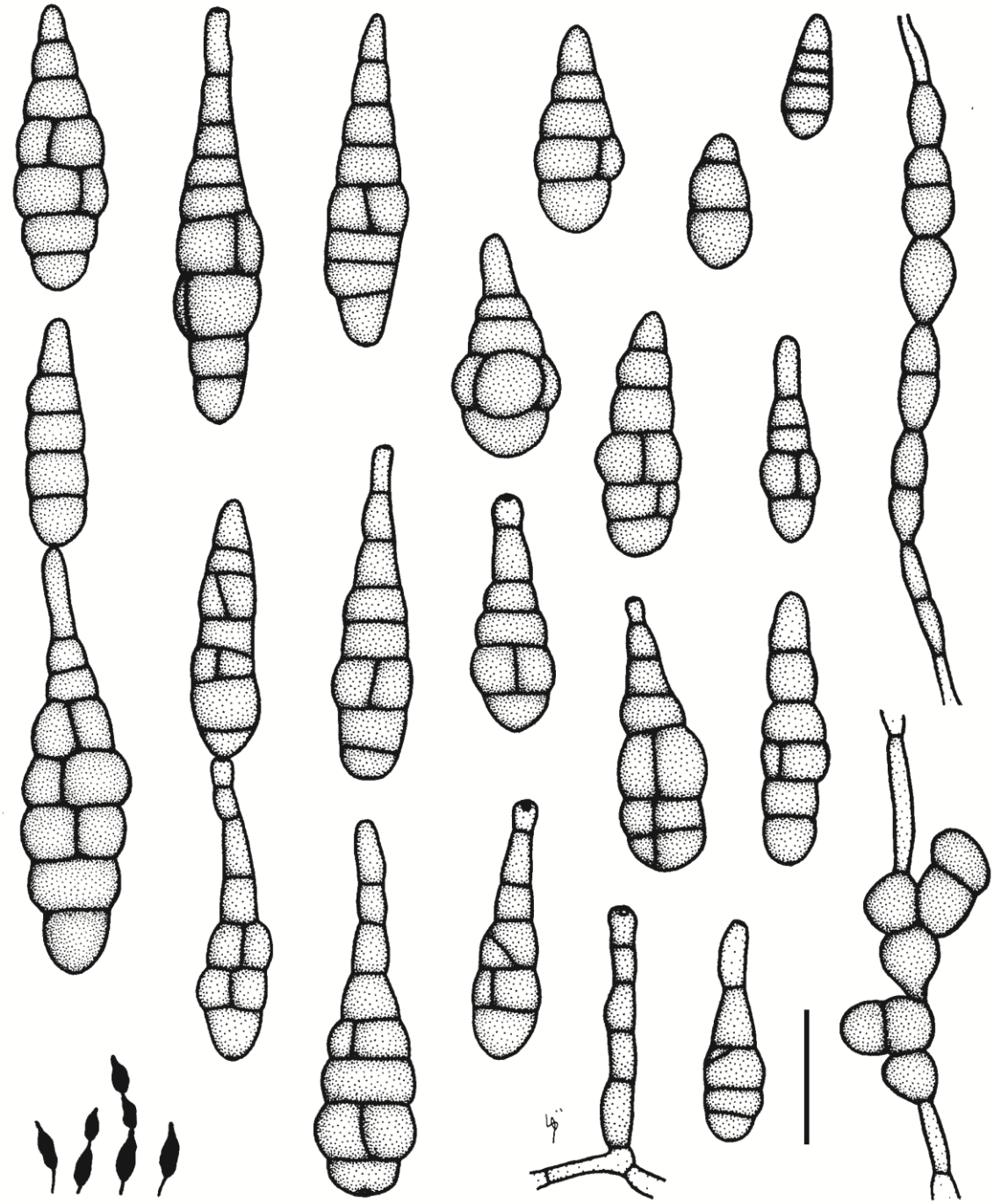

Fig. 5.30. Illustrations of *Alternaria japonica* (MAFF 246775). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on potato-carrot agar medium. Bar = 25 μm.

Fig. 5.31. Morphological features of Japanese isolates of *Alternaria japonica* (MAFF 246775) on potato-carrot agar medium. **A–E.** Conidia. **F.** Conidiophore. **G–I.** Chlamydospores. **J.** Culture on potato-dextrose agar medium (left = surface, right = reverse). **K.** Dried culture specimen ex MAFF 246775 (epitype: TNS-F-85453).

Epitype (**designated here**): **Japan**, Tokyo, Setagaya, from seeds of *Raphanus sativus* L., 24 Jul. 2000, *J. Nishikawa*, TNS-F-85453 (dried culture specimen of MAFF 246775) [MBT 385028], isoepitype MUMH 11696, culture ex-epitype MAFF 246775 = AC74.

Collections examined: **Japan**, Tokyo, Setagaya, from seeds of *Raphanus sativus* L., 24 Jul. 2000, *J. Nishikawa*, TNS-F-85453 (**epitype**), MUMH 11696 (**isoepitype**), living cultures AC73 and ex-epitype MAFF 246775 = MUCC 1622 = AC74; Shizuoka Prefecture, Kakegawa, on buds of *Brassica oleracea* var. *italica* Plenck, 7 Jun. 2010, *K. Takebayashi*, living culture AC96; ibid., on stem of *B. oleracea* var. *italica*, 7 Jun. 2010, *K. Takebayashi*, living culture AC97.

Morphological character on PCA medium: Conidia form as ex-epitype solitary or in short chains of 1–2, without lateral branches, measuring 20–84 µm in total length. Conidial bodies are ovoid to obclavate, ellipsoid, pale brown to brown and smooth, measuring 20–68 × 8–25 µm, with 2–7 transverse septa and 0–4 longitudinal septa, and are constricted at some transverse septa. Secondary conidiophores (false beaks) are usually short, 1–3 celled, unbranched, and measure 5–20 × 4–10 µm. Conidiophores are solitary, short, and narrow, measuring 18–80 × 4–6 µm. Intercalary chlamydospores frequently form both in air and submerged in agar substrate, either as single spores or in knots or chains, are brown to dark brown, and measure 10–21 × 8–16 µm.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching an average of 59.1 ± 2.9 mm in diam after 7 d at 25 °C, though there are variations among strains, and are rounded with white margins at the circumference. Aerial hypha are cottony, and vary in color from white or pale gray to grayish green, and reverse center dark green to black. Sporulation is sparse, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Brassicaceae*. There are also reported exceptions of *Carya illinoensis* (*Juglandaceae*), *Kalanchoe* sp. (*Crassulaceae*), *Oryza sativa* (*Poaceae*), *Sesamum indicum* (*Pedaliaceae*), and *Vigna unguiculata* (*Fabaceae*) serving as hosts.

Symptoms: Head rot and leaf spot like those caused by *A. brassicicola*, but caespituli appear sparsely on lesions.

Experimental host range: Strongly pathogenic to *Brassicaceae*, including *Diplotaxis* (*Brassiceae*), *Lobularia* (*Alysseae*), *Matthiola* (*Anchonieae*), and *Nasturtium* (*Cardamineae*); weakly pathogenic to *Eutrema* (*Eutremeae*), *Iberis* (*Iberideae*), and *Aubrieta* (*Arabideae*); non-pathogenic to *Capsella* (*Lepidieae*) and non-*Brassicaceae* plants (Table 5.5)

Distribution: Worldwide, including Asia (Japan, China, India, Korea, Nepal, etc.), Europe (Italy, Spain, Austria, etc.), North and Latin America (USA, Canada, Brazil, etc.), Africa (South Africa, Zimbabwe, Tunisia, etc.), and the Pacific (Australia, New Zealand, New Caledonia, etc.) (Yoshii 1941; Rao 1969; Ellis 1971; Richardson 1990; Tohyama & Tsuda 1990; Jasalavich 1995; Sharma & Tewari 1998; Yu 2001; Zhang 2003; Su *et al.* 2005; Simmons 2007; Gannibal & Gasich 2009; Ren *et al.* 2012; Bassimba *et al.* 2013; Woudenberg *et al.* 2013; Siciliano *et al.* 2017; Farr & Rossman 2018).

Distinctive features: Small conidia are either solitary or appear in short chains. Intercalary chlamydospores frequently form both in air and submerged in agar substrate. This species is widely pathogenic to *Brassicaceae*, but non- or weakly to *Eutrema*, *Aubrieta* and *Capsella*. It is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Notes: As for *A. nepalensis*, as described by Simmons (2007), it was clearly appropriate to synonymize it with *A*. *japonica* based on its conidial morphology, phylogenetic analysis, and its original source (from seeds of *Brassica* sp.).

Section *Panax* D.P. Lawr., Gannibal, Peever & B.M. Pryor, *Mycologia* **105**: 541. 2013. [MB 802308].

Woudenberg *et al.* (2013) assigned five species to this section, which is morphologically characterized by small to large conidia with blunt-tapered false beaks and broad conidiophores. Two species, *A*. *avenicola* and *A*. *photistica*, have a sexual morph (Simmons 1986, 2007). Only one species, *A*. *panax*, is distributed in Japan, and was described in the present study.

Alternaria panax Whetzel, in Whetzel & Rosenbaum, *Bull. U.S.D.A. Bur. Pl. Industr.* **250**: 11. 1912. [MB 121048]. **Fig. 5.32**.

≡ *Alternaria panax* Whetzel, in Cowles, *Science n. s.* **29**: 912. 1909, nom. nud.

≡ *Alternaria panacis* Whetzel, in Saccardo, *Syll. Fung.* **25**: 864. 1931, citing Rosenbaum & Zinnsmeister, *J. Agric. Res.* **5**: 181. 1915, orthographic variant (Art. 60.1). [MB 215071].

= *Macrosporium araliae* Dearn. & House, *Circ. N.Y. St. Mus.* **24**: 58. 1940, nom. inval. (no Latin; Art. 36) . [MB 287878].

= *Alternaria araliae* H.C. Greene, *Trans. Wisc. Acad. Sci.* **42**: 80. 1953. non *Alternaria araliae* sensu Deng *et al.*, *Mycol. Progr.* **14** (31): 4. 2015, inappropriate quotation. [MB 292406].

= *Alternaria actinophylla* J.W. Mille, *Fl. Dep. Agr., Div. Pl. Ind., Pl. Pathol. Circ.* **80**: 1969, nom. nud. (no Latin and type; Art. 36).

Type: **CUP** 4852 (not found in CUP database now).

Isotype: **USA**, New York, Fulton, on *Panax quinquefolius* L., 15 Jun. 1909, *H.H. Whetzel*, BPI 446440 ex CUP 4852; EGS07.074; BPI 803139 [MBT 119869].

Ex-type culture: unknown.

Collections examined: **Japan**, Tokyo, Ogasawara (Bonin Is.), Chichijima, on leaves of *Polyscias fruticosa* (L.) Harms, Jan. 2003, *T. Ono*, living culture AC18 = PFAlt1-1; ibid., on leaves of *Polyscias guilfoylei* (W. Bull) L. H. Bailey, Apr. 2003, *T. Ono*, living culture AC19 = PGAlt1; Tokyo, Ogasawara (Bonin Is.), Hahajima, on leaves of *P. fruticosa*, 28 Oct. 2011, *T. Sato*, MUMH 11686, living cultures MAFF 243161 and MAFF 243162.

Morphological character on V8 medium: Conidia commonly grow in chains of 2–7, without or rarely with lateral branches. They are yellowish brown to brown, smooth, oblong to long obclavate, with a blunt-tapered false beak, mostly straight and laterally symmetrical but occasionally excessively swollen. They are also often constricted at each transverse segment, ranging from 51–208 µm in total length. Conidial bodies range from 28–118 × 13–38 µm, with 4–13 transverse septa and up to 9 (often complicated) longitudinal septa; false beaks unbranched and are not filamentous, ranging from 9–110 × 3–9 µm, and are pale brown to brown. Conidiophores are broad, brown and unbranched, ranging from 55–145 × 7–10 µm.

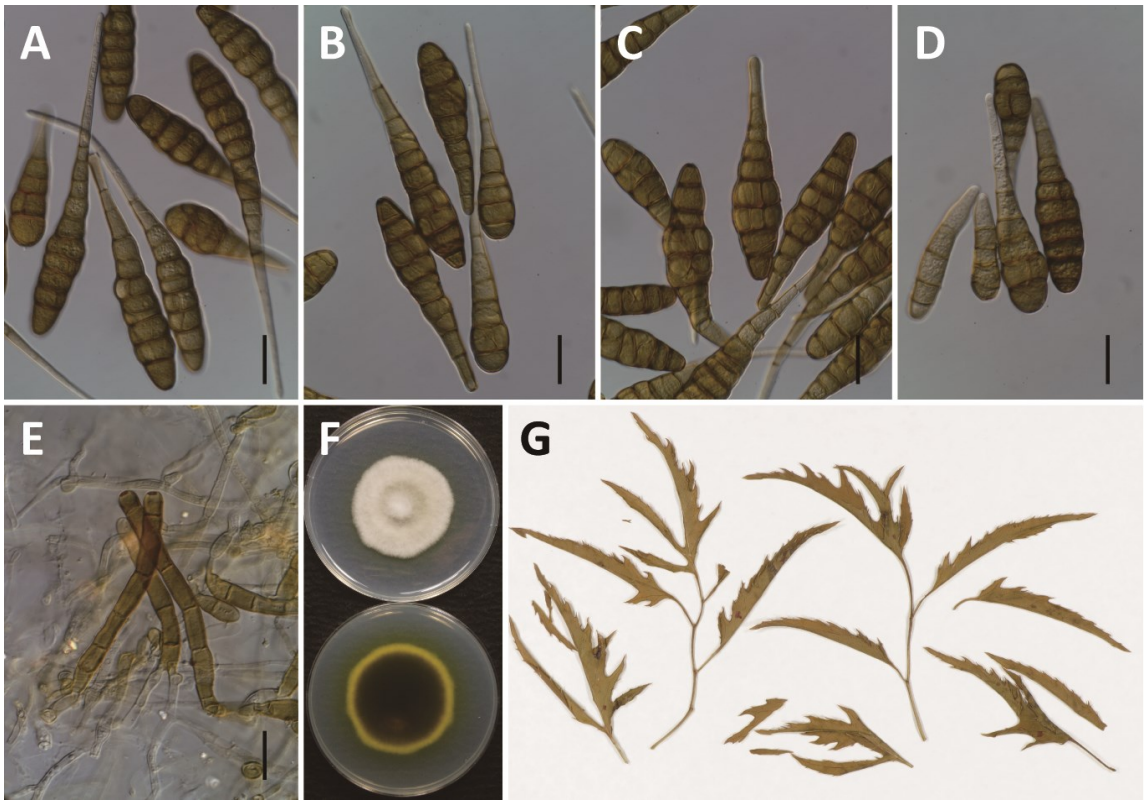

Fig. 5.32. Morphological features of Japanese isolates of *Alternaria panax* (MAFF 243161) on V8 juice agar medium. **A–D.** Conidia. **E.** Conidiophores. **F.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). **G.** Specimens of diseased leaves of *Polyscias fruticosa* (MUMH 11686).

Culture characteristics on PDA medium: Colonies are slow to moderate-growing, reaching an average of 46.5 ± 2.2 mm in diam after 7 d at 25 °C, and have a rounded circumference. Aerial hypha are cottony, white to pale gray, and reverse center dark green to black. Sporulation is sparse; it may release no pigment into the medium, or it may release bright yellow to reddish orange pigment.

Teleomorph: Not observed on RSA medium.

Natural host: *Araliaceae* (*Acanthopanax*, *Aralia*, *Brassaia*, *Dendropanax*, *Echinopanax*, *Fatsia*, *Kalopanax*, *Meryta*, *Panax*, *Plerandra*, *Polyscias*, *Pseudopanax*, *Schefflera*, and *Tupidanthus*).

Symptoms: Leaf and petiole spots on *Polyscias fruticosa*, appearing water-soaked to circular, brown, becoming enlarged and confluent, measuring 2–5 mm in diam.

Distribution: Japan (Bokura 1915; Ono 2004; Zhang *et al.* 2009), Korea (Yu 2001; Deng *et al.* 2010, 2013), China (Zhang 2003), New Zealand (Deng *et al.* 2013), the USA (Atilano 1983; Uchida *et al.* 1984; Woudenberg *et al.* 2013), Canada (Farr & Rossman 2018), and Italy (Garibaldi *et al.* 2004).

Distinctive features: Small and large conidia grow in short chains, with blunt-tapered false beaks. Colonies grown on PDA release either no pigment or a bright yellow to reddish orange pigment into the medium. This species is widely pathogenic to *Araliaceae*, and is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Notes: Deng *et al.* (2015) split *A*. *panax* into three species, *A*. *araliae*, *A*. *dendropanacis*, and *A*. *panax* (as *A*. *panacis*, orthographic variant), based on their culture characteristics, phylogeny (*Alt a 1*, *β-tubulin*, *tef1*, *gapdh*, and *rpb2*), and hosts. However, among these, *A*. *araliae* sensu Deng *et al.* was unrelated to the type material of *A*. *araliae* H.C. Greene (BPI 445904). Japanese isolates examined in the present study could not accommodate the definitions of these three species, which focused on pigment production and conidial morphology, as established by Deng *et al.* (2015).

Section *Porri* D.P. Lawr., Gannibal, Peever & B.M. Pryor, *Mycologia* **105**: 541. 2013. [MB 802309].

This section is the largest and, morphologically, the most confusable section. It was morphologically characterized by large spores that were usually non-catenated with

filamentous beaks and broad conidiophores, consists of 63 species as defined by Woudenberg *et al.* (2014). Among these, only twelve species are distributed in Japan (NIAS Genebank database of plant diseases in Japan: https://www.gene.affrc.go.jp/databases-micro_pl_diseases_en.php), and five species are described in the present study.

Alternaria crassa (Sacc.) Rands, *Phytopathology* **7**: 337. 1917. [MB 118901]. **Figs** 4.4, 4.5a; **5.33**.

≡ *Cercospora crassa* Sacc., *Michelia* **1** (1): 88. 1877. [MB 184395].

= *Cercospora daturae* Peck, *Rep.* (*Annual*) *New York State Mus. Nat. Hist.* **35**: 140. 1884. [MB 179162].

= *Macrosporium cookei* Sacc., *Syll. Fung.* **4**: 530. 1886. [MB 149467].

≡ *Macrosporium solani* Cooke, *Grevillea* **12**: 32. 1883. non *M. solani* Ellis & G. Martin, 1882.

≡ *Alternaria cookei* (Sacc.) Bremer, Işmen, Karel & Özkan & M. Özkan, *Istanbul Univ. Fak. Mecm., B* **13**: 42. 1948. [MB 284024].

= *Macrosporium daturae* Fautrey, in Lambottle & Fautrey, *Rev. Mycol.* (*Toulouse*) **16**: 76. 1894. [MB 193725].

≡ *Alternaria daturae* (Fautrey) Bubák & Ranoj., in Kobát & Bubák, *Fungi Imperf. Exsicc.* **14**: 694. 1911. [MB 416273].

= *Alternaria capsici* E.G. Simmons, *Mycotaxon* **75**: 84. 2000. [MB 467492].

Type: on leaves of *Datura stramonium* L. (details unknown; two specimens in **PAD**).

Lectotype: **PAD**, *Datura stramonium* L., S. [elva] '76. 10. (designated in Simmons 2000) [MBT 52700].

Epitype: **Cyprus**, Famagusta, on leaves of *Datura stramonium* L., Jan. 1936, *R.M. Nattrass*, CBS H-21744 [MBT 178115] (designated in Woudenberg *et al.* 2014), culture ex-epitype CBS 110.38.

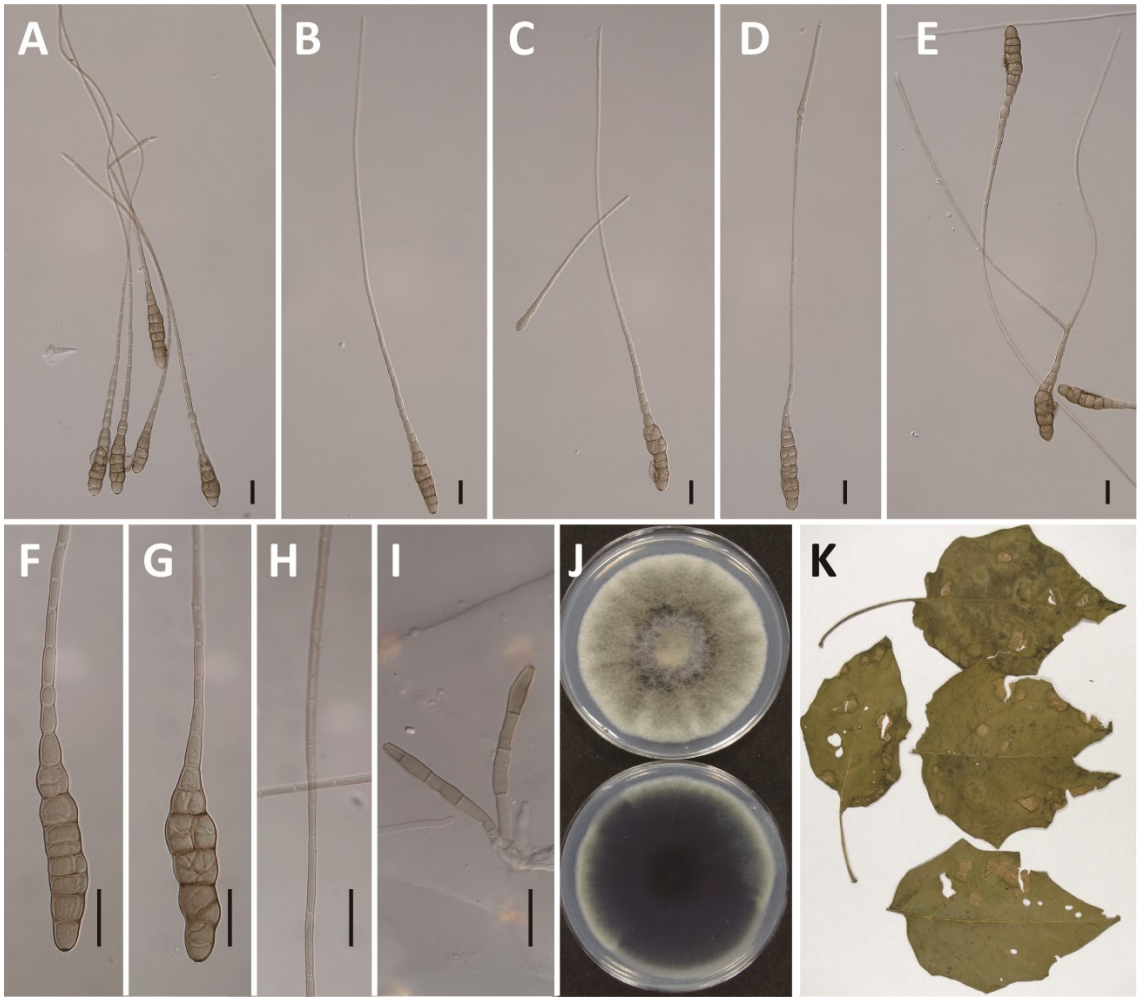

Fig. 5.33. Morphological features of Japanese isolates of *Alternaria crassa* (MAFF 243056) on V8 juice agar medium. **A–H.** Conidia with colored beaks. **I.** Conidiophores. **J.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). **K.** Symptoms on specimens of *Datura inoxia* (MUMH 11688). Bars (A–I) = 25 µm.

Collections examined: **Japan**, Tokyo, Kodaira, on leaves of *Datura stramonium* L., Jul. 2000, *J. Nishikawa*, living culture MAFF 243056 = AC4; ibid., on leaves of *Datura fastuosa* L., 20 Oct. 2012, *Ichinose et al.*, MUMH 11689 ex 12-M0180 (Hosei Univ.), living culture MUCC 2502 =

AC131; on leaves of *Datura inoxia* Mill., 15 Sep. 2012, *Ichinose et al.*, MUMH 11688 ex 12-M0099 (Hosei Univ.), living culture MUCC 2503 = AC132.

Morphological character on V8 medium: Conidia typically grow as solitary conidia, but may appear occasionally in chains of immature conidia, and reach 96–587 µm in total length. Conidial bodies are oblong to subcylindrical, measuring 30–101 × 6–23 µm, with 2–11 transverse and 0–8 longitudinal septa, and are pale brown with a surface smooth. False beaks are usually unbranched but are very occasionally branched, straight, often elongated, multiseptated, subhyaline to pale brown, measure 66–515 × 3–6 µm, and inconspicuously border the conidial body. Conidiophores are short and broad, measuring 25–58 × 5–8 µm.

Culture characteristics on PDA medium: Colonies fast-growing, reaching an average of 80 ± 1.8 mm in diam after 7 d at 25 °C. They are slightly rounded, with white margins at the circumference. Aerial hypha are cottony, grayish green to black, and reverse center dark green to black. Sporulation is sparse, and pigment is never released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Datura* spp. (including *Brugmansia*), *Capsicum annuum*, *Nicandra physalodes*, *Petunia* × *hybrida*, and *Solanum nigrum* (*Solanaceae*).

Symptoms: Leaf spots appear on *Datura stramonium*, and are vein-limited circular to irregular, straw-yellow to pale brown with a gray center, distinct at their borders, and are scattered, but become enlarged and confluent.

Host range: *Datura* (tribe *Datureae*, *Solanoideae*) and *Capsicum* (tribe *Capsiceae*, *Solanoideae*), and occasionally on genera of the other tribe or subfamily in *Solanaceae* (Table 4.1).

Distribution: Worldwide, including Asia (Japan, Taiwan, China, India, Israel, etc.), Europe (Latvia, Poland, Cyprus, etc.), North and Latin America (USA, Cuba, El Salvador, and Venezuela), Africa (Kenya, South Africa, Tanzania, etc.), and the Pacific (Australia and New Zealand) (Sawada

1944; Rao 1969; Richardson 1990; Crous *et al.* 2000; Zhang 2003; Woudenberg *et al.* 2014; Ichinose *et al.* 2015; Farr & Rossman 2018).

Distinctive features: Large-spored species with filamentous but clear false beaks, which are usually unbranched, colored, significantly elongated, and often exceed 4 μm in width. Conidial bodies are pale brown, with longitudinal septa in common. Colonies on PDA medium released no pigment. This species is pathogenic to *Datura* and *Capsicum*, and is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Notes: Woudenberg *et al.* (2014) synonymized *A*. *capsici* based on the combined phylogeny of its ITS, *gapdh*, *tef1*, *rpb2*, and *Alt a 1* sequences, and then inoculation tests conducted by Nishikawa & Nakashima (2013) also supported this taxonomic classification. In addition, Nishikawa & Nakashima (2013) suggested that *A*. *daturicola* was also a probable synonym.

Alternaria cucumerina (Ellis & Everh.) J.A. Elliott, *Amer. J. Bot.* **4**: 472. 1917. [MB 100434]. **Fig. 5.34**.

≡ *Macrosporium cucumerinum* Ellis & Everh., *Proc. Acad. Nat. Sci. Philadelphia* **47**: 440. 1895. [MB 194117]

≡ *Alternaria cucumerina* var. *cucumerina* (Ellis & Everh.) J.A. Elliott, *Amer. J. Bot.* **4**: 472. 1917. [MB 427399].

= *Alternaria loofahae* E.G. Simmons & Aragaki, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 316. 2007. [MB 505008].

Type: **USA**, New Mexico, Las Cruces, on leaves of *Cucumis melo* L., Aug. 1895, *E.O. Wooton*. (not specified).

Lectotype: **USA**, New Mexico, Las Cruces, on leaves of *Cucumis melo* L., Aug. 1895, *E.O. Wooton*, **PH** (designated in Simmons 2007).

Ex-type culture: Unknown.

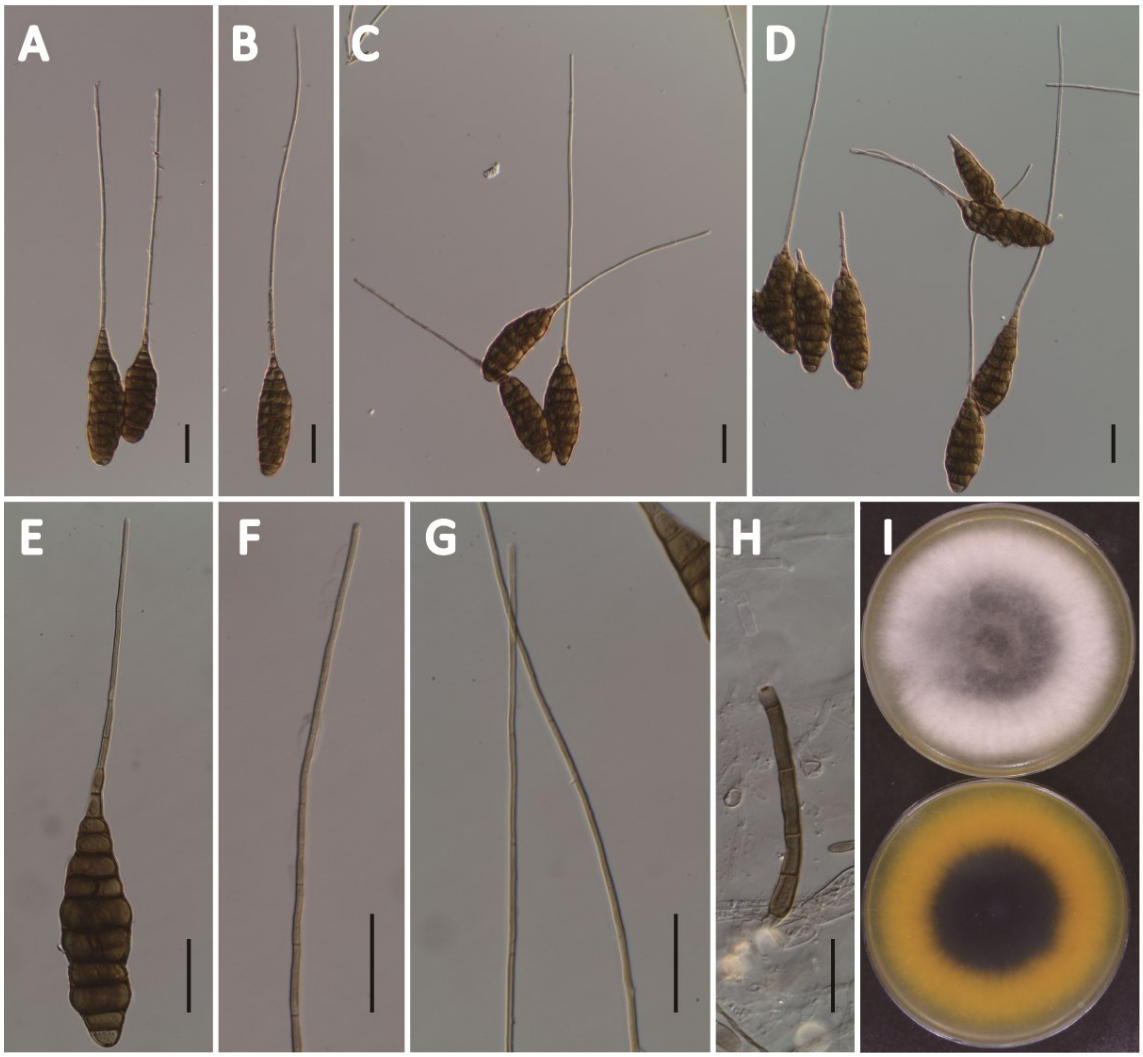

Fig. 5.34. Morphological features of Japanese isolates of *Alternaria cucumerina* (AC106) on V8 juice agar medium. **A–E.** Conidia. **F, G.** Colored beaks. **H.** Conidiophore. **I.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). Bars (A–H) = 25 µm.

Collections examined: **Japan**, Niigata Prefecture, Sado, on leaves of *Cucurbita maxima* Duchesne, 27 Jul. 2010, *Y. Makizumi*, living culture AC105; ibid., 30 Jul. 2010, *Y. Makizumi*, living culture AC106.

Morphological character on V8 medium: Conidia are usually solitary, but will occasionally form in chains of 2, and measure 56–411 µm in total length. Conidial bodies are subcylindrical to broadly obclavate and oblong, measuring 36–106 x 13–28 µm, with 4–15 transverse and 2–12 (often complicated) longitudinal septa, and are brown to dark brown with a smooth surface. Filamentous beaks are almost straight and are unbranched, measuring 16–305 × 1–2 µm. They are pale brown and conspicuously distinguishable, bordering the conidial body. Conidiophores are moderately long and broad, ranging from 69–109 × 5–7 µm.

Culture characteristics on PDA medium: Colonies are fast-growing with a rounded circumference, reaching an average of 87 ± 0.7 mm in diam after 7 d at 25 °C. Aerial hypha are cottony, white to pale gray, and reverse center dark green to black. Sporulation is sparse, and usually no pigment is released into the medium, although occasionally a bright yellow to pale orange pigment may be released.

Teleomorph: Not observed on RSA medium.

Natural host: *Cucurbitaceae* (*Benincasa*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria*, and *Luffa*), as well as occasionally reported on *Asimina* (*Annonaceae*), *Cyamopsis* and *Phaseolus* (*Fabaceae*).

Symptoms: Leaf spots appear on *Cucurbita maxima*, and are dark brown to black with grayish eye at center, subcircular to angular with a distinct border, 1–5 mm in diam, becoming confluent.

Distribution: Worldwide, including Asia (Japan, China, Korea, India, etc.), Europe (Russia, UK, etc.), North and Latin America (USA, Mexico, Haiti, etc.), Africa (South Africa, Zimbabwe, Libya, etc.), and the Pacific (Australia and New Zealand) (Benjamin & Slot 1969; Ellis 1971; Yu 2001; Zhang 2003; Gannibal 2011; Woudenberg *et al.* 2014; Farr & Rossman 2018).

Distinctive features: Large-spored species with filamentous colored beaks, which are usually unbranched and do not exceed 3 µm in width. Conidial bodies are broadly obclavate to oblong,

often with complicated longitudinal septa. Colonies on PDA often release yellow to pale orange pigment into the medium. This species is phylogenetically recognizable via its *gapdh*, *tef1*, *Alt a 1*, and *act* sequences.

Alternaria dauci (J.G. Kühn) J.W. Groves & Skolko, *Canad. J. Res., Sect. C, Bot. Sci.* **22** (5): 222. 1944. [MB 284025]. **Fig. 5.35**.

≡ *Sporidesmium exitiosum* var. *dauci* J.G. Kühn, *Hedwigia* **1**: 91. 1855. [MB 416592].

≡ *Polydesmus exitiosus* var. *dauci* (J.G. Kühn) J.G. Kühn, *Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung*: 165. 1858. [MB 137353].

≡ *Macrosporium dauci* (J.G. Kühn) Rostr., *Tidsskr. Landoekon. ser. 5*, **7**: 385. 1888. [MB 416163].

= *Macrosporium carotae* Ellis & Langl., *J. Mycol.* **6** (1): 36. 1890. [MB 147381].

≡ *Alternaria carotae* (Ellis & Langl.) J.A. Stev. & Wellman, *J. Wash. Acad. Sci.* **34**: 263. 1944. [MB 284021].

≡ *Alternaria brassicae* var. *dauci* (J.G. Kühn) Lindau, *Rabenh. Krypt.-Fl.,* Edn 2 (*Leipzig*) **1** (9): 260. 1908. [MB 137491]. non *Alternaria brassicae* var. *dauci* (J.G. Kühn) P.C. Bolle, *Meded. Phytopathol. Lab.* "*Willie Commelin Scholten*" **7**: 42. 1924, later isonym.

≡ *Alternaria dauci* f. *dauci* (J.G. Kühn) J.W. Groves & Skolko, *Canad. J. Res., Sect. C, Bot. Sci.* **22** (5): 222. 1944. [MB 429113].

≡ *Alternaria porri* f. sp. *dauci* (J.G. Kühn) Neerg, *Danish species of Alternaria & Stemphylium*: 252. 1945. [MB 351635].

= *Alternaria poonensis* Ragunath, *Mycopathol. Mycol. Appl.* **21**: 315. 1963. [MB 326068].

Lectotype: **B**, slide glass specimen of *Sporidesmium exitiosum* var. *dauci* Kühn, Gross Krausche p. Bunzlau, Jul., *Kühn*. (designated in Simmons 1995b; appeared to be lost according to Woudenberg *et al.* 2014).

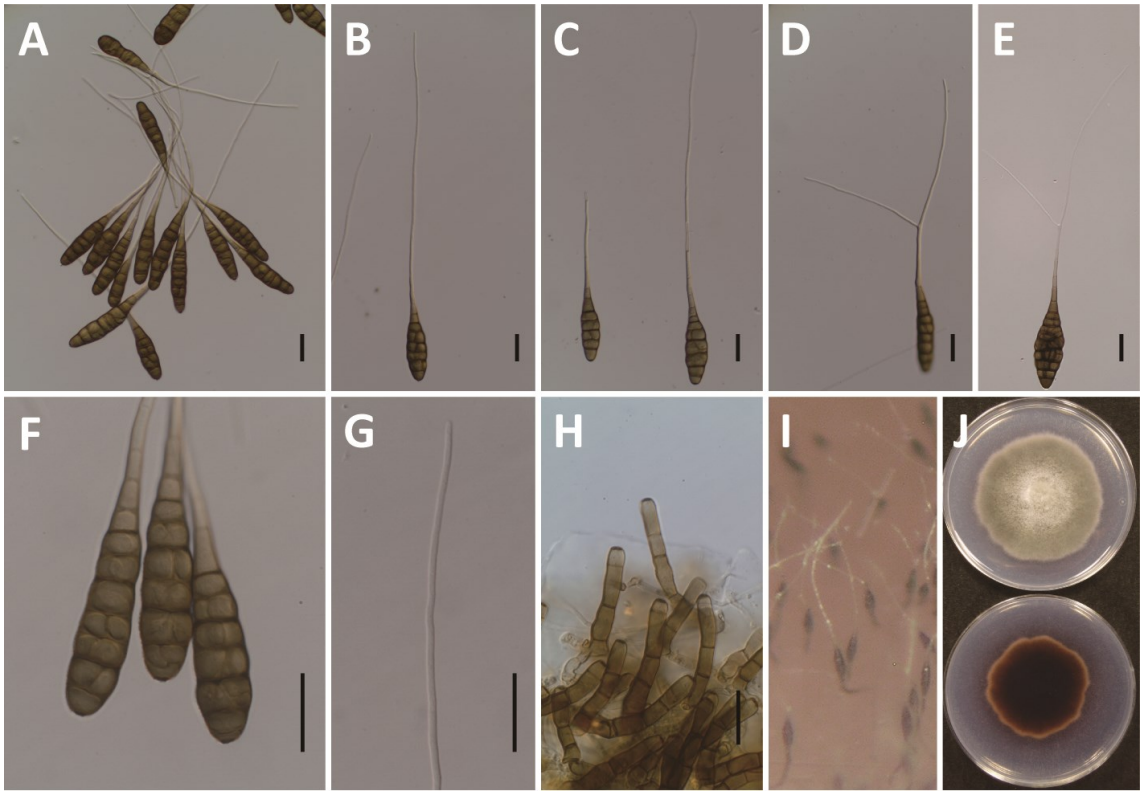

Fig. 5.35. Morphological features of Japanese isolates of *Alternaria dauci* (AC8) on V8 juice agar (V8) medium. **A–G.** Conidia with filamentous beaks. **H.** Conidiophore. **I.** Sporulation on surface of V8 medium. **J.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). Bars (A–H) = 25 µm.

Neotype: **Italy**, from seed of *Daucus carota* L., Sep. 1937, *P. Neergaard*, CBS H-21745 (designated in Woudenberg *et al.* 2014) [MBT 178116], culture ex-neotype CBS 111.38.

Collections examined: **Japan**, Shizuoka Prefecture, Kakegawa, on leaves of *Daucus carota* L., Nov. 1998, *K. Takebayashi*, living cultures AC8 and AC9.

Morphological character on V8 medium: Commonly forms solitary conidia measuring 152–448 µm in total length. Conidial bodies are oblong to broadly obclavate, measuring 52–100 ×

13–31 µm, with 5–11 transverse and 0–8 longitudinal septa, which sometimes remain as distosepta-like structures, and they are brown to dark brown with a smooth surface. Filamentous beaks are straight and elongated, hyaline to subhyaline, unbranched to branched once, and inconspicuously border the conidial body, measuring 100–368 × 1–3 µm. Conidiophores are moderately long and broad, ranging from 36–94 × 6–8 µm.

Culture characteristics on PDA medium: Colonies are slow to moderate-growing, reaching an average of 53.6 ± 3.3 mm in diam after 7 d at 25 °C, though there are variations among strains, and are almost rounded with white margins at circumference. Aerial hypha are cottony, grayish green to dark green, and reverse center dark green to black. Sporulation is sparse, and usually red to reddish brown pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Daucus*, *Coriandrum*, *Apium* (*Apiaceae*), and *Cichorium* (*Asteraceae*), as well as some recorded cases infecting non-*Apiaceae* families under heterogeneous names as forma speciales of *A*. *dauci*.

Symptoms: Spots appear on the leaves and petioles of *Daucus*, and are circular to subcircular with distinct margins, measuring 1–3 mm in diam, which become confluent, resulting in severe leaf blight and causing significant economic losses.

Distribution: Worldwide, including Asia (Japan, China, India, Korea, Nepal, Israel, etc.), Europe (Denmark, Netherlands, UK, Germany, Italy, Turkey, Russia, Portugal, etc.), North and Latin America (USA, Puerto Rico, Haiti, Nicaragua, etc.), Africa (Guinea, South Africa, etc.), and the Pacific (New Zealand, etc.) (Goto 1927; Kranz 1963; Benjamin 1969; Rao 1969; Richardson 1990; Crous *et al.* 2000; Yu 2001; Zhang 2003; Soylu *et al.* 2004; Lopes & Martins 2008; Delgado 2011; Woudenberg *et al.* 2014; Farr & Rossman 2018; Ozkilinc *et al.* 2018; Poudel & Zhang 2018).

Distinctive features: Conidial bodies are oblong to obclavate, with hyaline filamentous beaks that often have a single branch. The conidial morphology of the species is indistinguishable from those of *A. porri*, but differs in length and width (usually not exceeding 3 µm), and more frequently in their longitudinal septa. Its growth rate on PDA is clearly slower, and colonies produce red pigment. This species is pathogenic to *Daucus* and some other species in *Apiaceae* (Boedo *et al.* 2012), and is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Alternaria porri (Ellis) Cif., *J. Dept. Agric. Porto Rico* **14** (1): 30. 1930. [MB 215273]. **Figs 5.36**, **37**.

≡ *Macrosporium porri* Ellis, in Cooke and Ellis, *Grevillea* **8** (45): 12. 1879. [MB 243861].

≡ *Alternaria porri* (Ellis) Sawada, *Rep. Dept. Agric. Gov. Res. Inst. Formosa* **61**: 92. 1930, later isonym.

≡ *Alternaria porri* (Ellis) Neerg., *Aarsberet. J. E. Ohlsens Enkes Plantepat. Lab.* **3**: 5. 1938, later isonym.

= *Alternaria allii* Nolla, *Phytopathology* **17**: 118. 1927.

= *Alternaria vanuatuensis* E.G. Simmons & C.F. Hill, *CBS Biodiversity Ser.* (*Utrecht*) **6**: 260. 2007.

Type: on *Allium porrum* L. (holotype not specified).

Lectotype: **USA**, New Jersey, Newfield, on leaves of *A. porrum*, Sep. 1878, *Ellis*, **NY**. (designated in Simmons 2007).

Epitype: **USA**, New York, Orange County, from leaf of *Allium cepa* L., 1996, *M.J. Yáñes* Morales, CBS H-21746 [MBT 178117] (designated in Woudenberg *et al.* 2014), culture ex-epitype CBS 116699 = EGS48.152.

Collections examined: **Japan**, Shizuoka Prefecture, Kakegawa, on leaves of *Viola × wittrockiana* Gams, 24 Dec. 2003, *J. Nishikawa*, living culture AC2; ibid., on leaves of *Calibrachoa* sp., 23 Apr. 2004, *J. Nishikawa*, MUMH 11670 and MUMH 11699, living culture

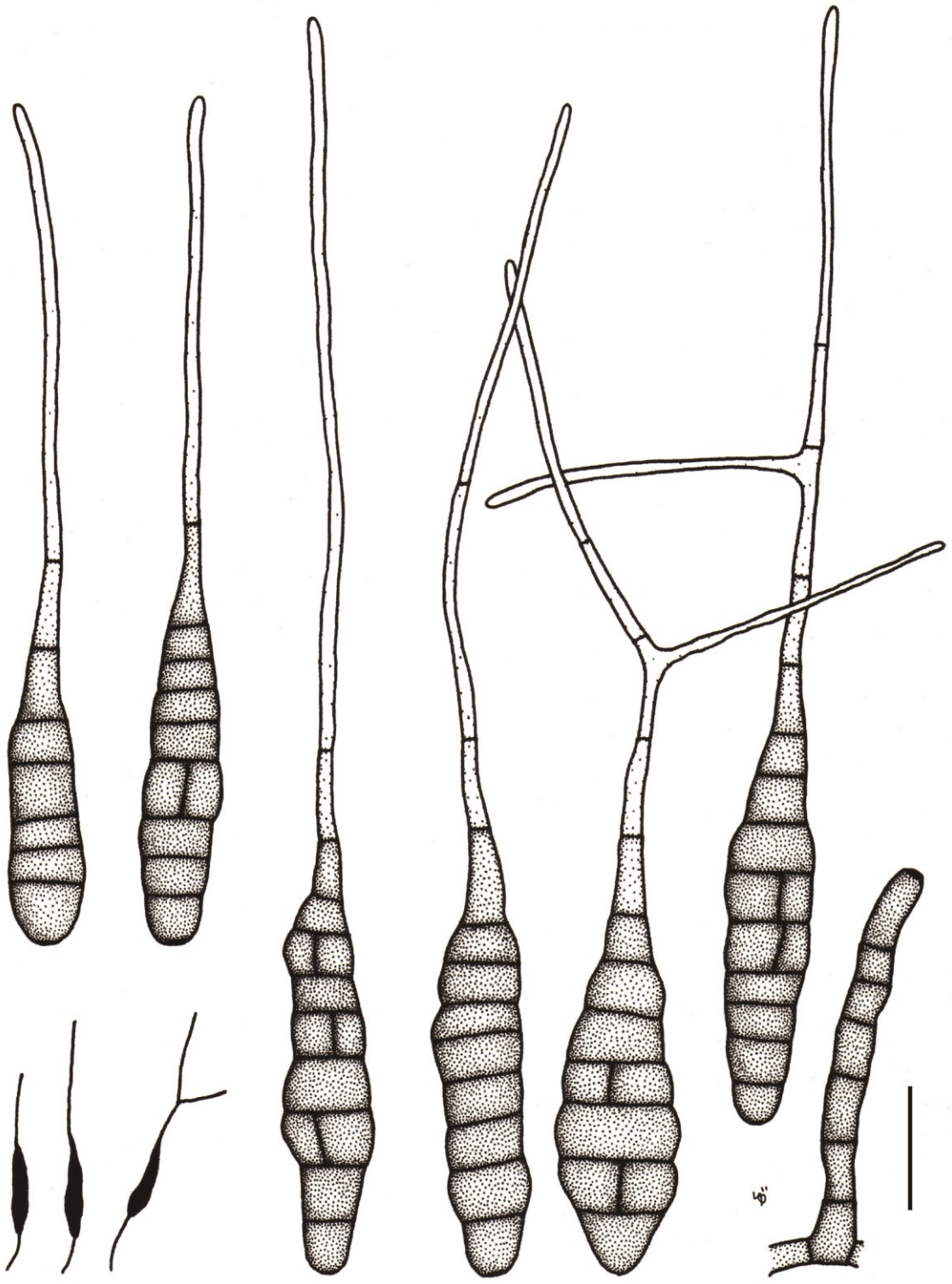

Fig. 5.36. Illustrations of *Alternaria porri* (AC6). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on V8 juice agar medium. Bar = 25 µm.

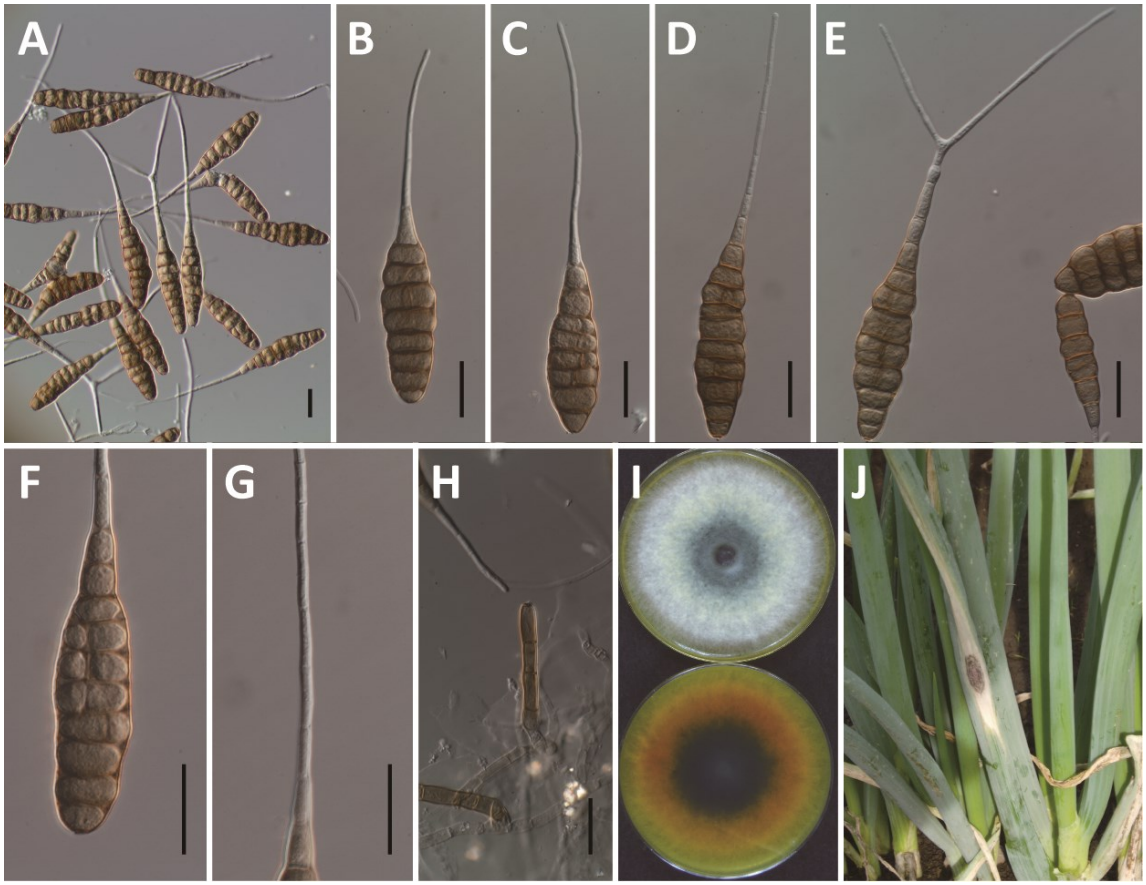

Fig. 5.37. Morphological features of Japanese isolates of *Alternaria porri* (AC14) on V8 juice agar medium. **A–G.** Conidia with filamentous beaks. **H.** Conidiophores. **I.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). **J.** Natural symptoms on *Allium fistulosum*. Bars (A–H) = 25 µm.

AC6; on leaves of *Allium fistulosum* L., 7 Oct. 2004, *J. Nishikawa*, MUMH 11673, living culture AC14; Saitama Prefecture, on leaves of *A. fistulosum*, 1 Nov. 2004, *J. Nishikawa*, living culture AC15; Gunma Prefecture, Takasaki, on leaves of *A. fistulosum*, 16 Mar. 2005, *J. Nishikawa*, living cultures AC16 and AC17; Shizuoka Prefecture, Kakegawa, on leaves of *A. fistulosum*, 4 Jun. 2005, *J. Nishikawa*, living culture AC24; Gunma Prefecture, Tomioka, on leaves of *A. fistulosum*, 6 Oct. 2006, *J. Nishikawa*, MUMH 11677, living culture MUCC 1698 = AC30; Chiba Prefecture, Mobara, on leaves of *A. fistulosum*, 24 Oct. 2006, *J. Nishikawa*, living culture AC32; Shizuoka

Prefecture, Kakegawa, from seeds of *Eustoma exaltatum* (L.) Salisb. ex G. Don subsp. *russellianum* (Hook.) Kartesz, 20 Mar. 2007, *Y. Makizumi*, MUMH 11692, living culture AC35; Tokyo, Setagaya, from seeds of *A. fistulosum*, 7 Jul. 2001, *J. Nishikawa*, living culture AC68; Shizuoka Prefecture, Kakegawa, on *A. fistulosum*, 16 Mar. 2012, *J. Nishikawa*, living culture AC93.

Morphological character on V8 medium: Conidia solitary in common, ranging 75–351 µm in total length. Conidial bodies are subcylindrical to oblong, pale brown to brown, have a smooth surface, and range from 38–114 × 10–26 µm with 3–12 transverse and 0–5 longitudinal septa, which sometimes remain as distosepta-like structures. Filamentous beaks are straight to slightly curved, hyaline, range from 30–248 × 2–4 µm, and are sometimes unbranched and often branched 1–2 times, and inconspicuously border the conidial body. Conidiophores are moderately long and broad, ranging from 35–139 × 6–11 µm. Conidial morphology on lesions is similar to those observed when grown on V8 medium, though they are usually short-beaked.

Culture characteristics on PDA medium: Colonies are fast-growing, reaching an average of 78.6 ± 1.1 mm in diam after 7 d at 25 °C, and are rounded sometimes having white margins at circumference. Aerial hypha are cottony, white to grayish green, and reverse center dark green, usually with bright yellow to orange or reddish brown pigment released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Allium* spp. (*Amaryllidaceae*), are the most common hosts, although there are reports of the following serving as occasional hosts: *Acalypha* (*Euphorbiaceae*), *Apium* (*Apiaceae*), *Calendula*, *Gerbera*, and *Tagetes* (*Asteraceae*), *Clarkia* (*Onagraceae*), *Dichondra* and *Ipomoea* (*Convolvulaceae*), *Gossypium* and *Mucuna* (*Malvaceae*), *Peganum* (*Nitrariaceae*), *Scabiosa* (*Dipsacaceae*), and *Solanum* (*Solanaceae*).

Symptoms: Leaf spots on *A. fistulosum* are circular to long elliptical, distinct sooty spots, often with purple-stained appearance, measuring 7–50 mm in diam. Caespituli were frequently observed on lesions.

Experimental host range: Pathogenic to *Allium* spp. (*Amaryllidaceae*), but not to leaves of *Calibrachoa*, *Capsicum*, *Petunia*, *Solanum*, and *Nicotiana* (data not shown).

Distribution: Worldwide, including Asia (Japan, China, India, Korea, etc.), Europe (Bulgaria, Denmark, etc.), North and Latin America (USA, Nicaragua, Puerto Rico, Haiti, etc.), Africa (South Africa, etc.), and the Pacific (Australia, etc.) (Yoshii 1929a; Rao 1969; Ellis 1971; Stevenson 1975; Richardson 1990; Aveling & Naude 1992; Koike & Henderson1998; Crous *et al.* 2000; Yu 2001; Zhang 2003; Hall *et al.* 2007; Simmons 2007; Delgado 2011; Woudenberg *et al.* 2013, 2014; Farr & Rossman 2018).

Distinctive features: Large-spored species with hyaline filamentous beaks, which are often branched and exceeded 3 µm in width. Conidial bodies are subcylindrical, with relatively fewer longitudinal septa. Colonies grown on PDA medium released bright yellow to reddish brown pigment. This species is pathogenic to *Allium*, and is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Notes: Woudenberg *et al.* (2014) recognized *A*. *allii*, which also defined a morphospecies having multiple branched beaks (Simmons 2007), as a distinct taxon based on the combined phylogeny of its ITS, *gapdh*, *tef1*, *rpb2*, and *Alt a 1* sequences. However, morphological observations and phylogenetic analysis conducted in the present study did not clearly support this species as a unique *Allium* pathogen (Fig. 5.2). Therefore, we regarded *A*. *allii* as a synonym of *A*. *porri* here. This species is also ubiquitous, and frequently found on non-host plants.

Alternaria zinniae H. Pape ex M.B. Ellis, *Mycol. Pap.* **131**: 22. 1972. [MB 284044]. **Fig. 5.38**.

≡ *Alternaria zinniae* H. Pape, *Angew. Bot.* **24**: 61. 1942, nom. inval. (Art. 36.1). [MB 284043].

Type: holotype specimen not specified.

Lectotype: **USA**, New York, Ithaca, on *Zinnia elegans* Jacq., 28 Sep. 1942, *A.W. Dimock*, IMI 1037 (designated in Simmons 2007; as holotype in Simmons 1997).

Ex-type culture: unknown.

Collections examined: **Japan**, Nagano Prefecture, Tomi, on leaves of *Zinnia hybr.*, 6 Jul. 2007, *J. Nishikawa*, MUMH 11680, living culture AC44; Nagano Prefecture, Azumino, on leaves of *Zinnia hybr.*, Aug. 2010, *Y. Makizumi*, living culture AC107; Shizuoka Prefecture, Kakegawa, on *Zinnia elegans* Jacq., 16 Mar. 2011, *Y. Makizumi*, living culture AC108; Nagano Prefecture, Azumino, on *Z. elegans*, 31 May 2011, *Y. Makizumi*, living culture AC109.

Morphological character on V8 medium: Conidia are usually solitary, but occasionally appear in chains of 2, measuring 109–318 µm in total length. Conidial bodies are subcylindrical to oblong, measuring 74–119 × 20–33 µm, with 9–16 transverse and 6–14 (often complicated) longitudinal septa. They are olive brown to brown, and have a smooth to minutely verrucose surface. Filamentous beaks are almost straight, unbranched, pale brown, are conspicuously distinguishable and border the conidial body, measuring 33–213 × 1–3 µm. Conidiophores are moderately long and broad, ranging from 60–183 × 6–8 µm.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching an average of 60.4 ± 5.6 mm in diam after 7 d at 25 °C, though there are variations among strains. They are almost rounded, with white margins at the circumference. Aerial hypha are cottony, white to pale gray, and reverse center dark green to black, often with yellow to pale orange pigment released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: Usually *Zinnia* and the other asteraceous plants (*Ageratum*, *Bidens*, *Blumea*, *Calendula*, *Callistephus*, *Carthamus*, *Coreopsis*, *Cosmos*, *Dahlia*, *Echinops*, *Eclipta*, *Eupatorium*, *Gaillardia*, *Galinsoga*, *Helianthus*, *Parthenium*, *Rudbeckia*, *Sphaeranthus*, *Spilanthes*, *Tagetes*, *Volutaria*, and *Xanthium*). Records suggest that it may also infect *Impatiens* (*Balsaminaceae*),

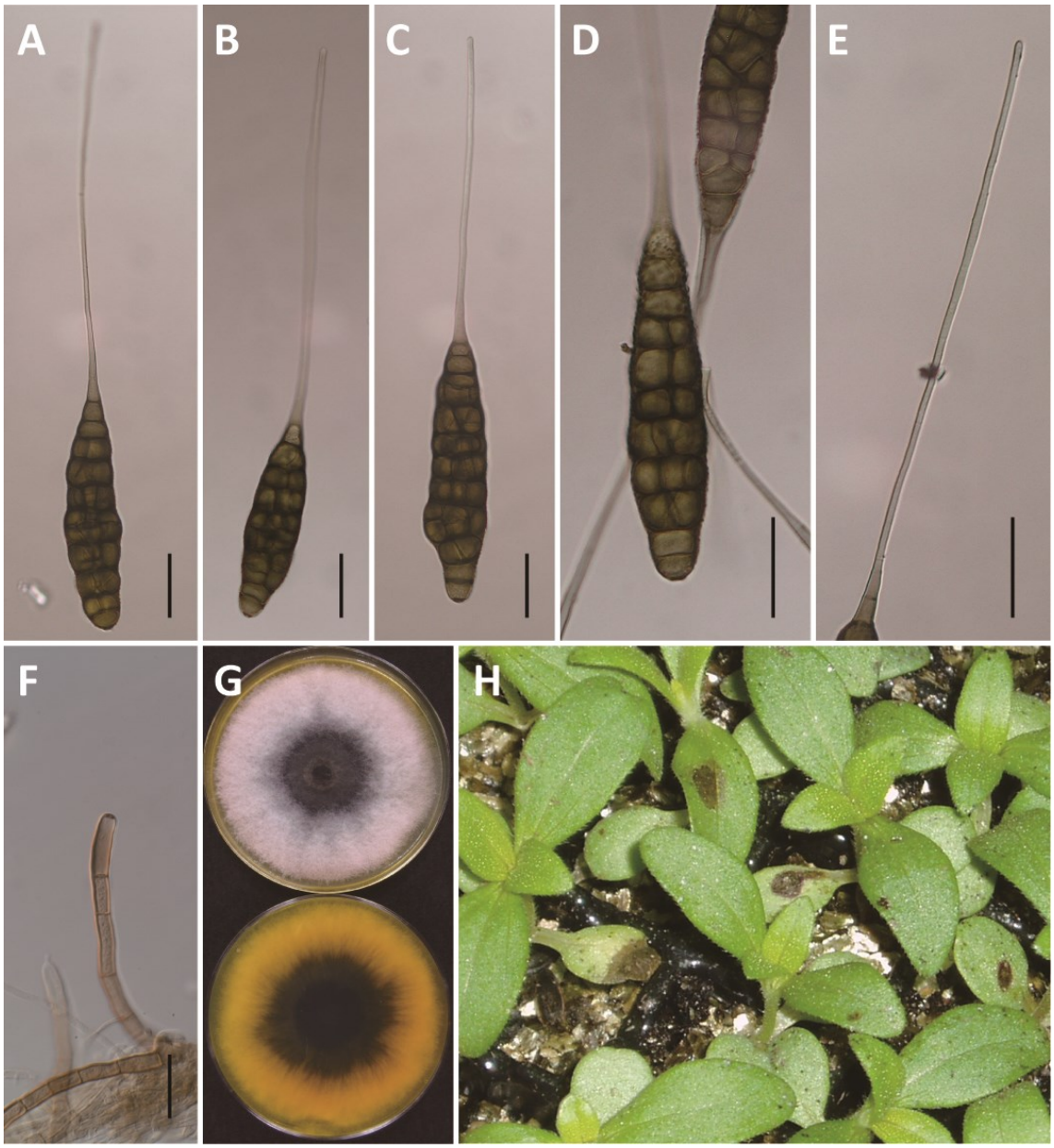

Fig. 5.38. Morphological features of Japanese isolates of *Alternaria zinniae* (AC44) on potato-carrot agar medium. **A–E.** Conidia. **F.** Conidiophores. **G.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). Bars (A–F) = 25 µm.

and *Nicotiana* (*Solanaceae*).

Symptoms: Leaf spots on seedlings of *Zinnia* are brown, circular to irregular, measuring 5–10 mm in diam, becoming enlarged and confluent.

Distribution: Worldwide, including Asia (Japan, India, China, Pakistan, Korea, etc.), Europe (Hungary, Denmark, Austria, Netherlands, Italy, UK, etc.), North and Latin America (Canada, USA, etc.), Africa (Guinea, South Africa, etc.), and the Pacific (New Zealand, etc.) (Kranz 1963; Rao 1969; Ellis 1976; Richardson 1990; Simmons 1997; Crous *et al.* 2000; Yu 2001; Zhang 2003; Woudenberg *et al.* 2014; Farr & Rossman 2018).

Distinctive features: Large-spored species with filamentous colored beaks, which are usually unbranched and do not exceed 3 µm in width. Conidial bodies are oblong, often with complicated longitudinal septa. Colonies grown on PDA medium sometimes release yellow to pale orange pigment into the medium. This species is phylogenetically recognizable via its *gapdh*, *tef1*, *Alt a 1*, and *act* sequences.

Notes: Since there are a wide range of records listing *Asteraceae* as a host, further pathological studies within *Asteraceae*, besides *Zinnia*, are needed to characterize the species.

Section *Pseudoulocladium* Woudenb. & Crous, *Stud. Mycol.* **75**: 201. 2013. [MB 803744].

The four species assigned to this section are characterized by simple or branched, short, and geniculate conidiophores, and catenation of mostly 3-septate conidia (Woudenberg *et al.* 2013). However, morphological differences between these species still have not been defined.

Alternaria chartarum Preuss, *Bot. Zeitung* **6**: 412. 1848. [MB 205475]. **Fig. 5.39**.

≡ *Alternaria chartarum* f. *chartarum* Preuss, *Bot. Zeitung* **6**: 412. 1848. [MB 427398]

≡ *Sporidesmium polymorphum* var. *chartarum* (Preuss) Cooke, *Fungi Brit. Exs., ser. 2*: 329. 1875. [MB 155155].

≡ *Ulocladium chartarum* (Preuss) E.G. Simmons, *Mycologia* **59** (1): 88. 1967. [MB 340658].

= *Alternaria stemphylioides* Bliss, *Mycologia* **36** (5): 538. 1944. [MB 284041].

≡ *Alternaria chartarum* f. *stemphylioides* (Bliss) P. Joly, *Encycl. Mycol.* (*Paris*) **33**: 161. 1964. [MB 349007].

Type: Germany, Hoyerswerda, **B**, on paper, *Preuss*, Klotzsch's Herb. vivum mycol. no. 1284.

Epitype: **Canada**, Saskatchewan, from *Populus* sp., Jul. 1957, *S.J. Hughes*, CBS H-19059 = DAOM 59616b [MBT 138375] (designated in de Hoog & Horré 2002), culture ex-epitype CBS 200.67 = ATCC 18044 = IMI 124943 = MUCL 18564 = QM 8328.

Collection examined: **Japan**, Tokyo, Setagaya, from seeds of *Capsicum annuum* L., 8 Dec. 2000, *J. Nishikawa*, living culture AC85.

Morphological character on PCA medium: Conidiophores are solitary and relatively short, measuring 18–95 × 3–5 µm. They are pale brown to brown, with polytretic pores at the apex and 2–4 geniculate bends, frequently proliferating at the upper nodes. Conidia grow in short chains of 3–8, frequently with lateral branches, are brown to dark brown, ellipsoid to obclavate, and are smooth to roughened, measuring 13–29 × 8–15 µm, with 1–4 (mostly 3) transverse and 0–4 longitudinal septa. Secondary conidiophores (false beaks) appear at the apical end and median of conidium, and are short, mostly single-celled, and sometimes proliferate and branched.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching 60.8 ± 3.1 mm in diam after 7 d at 25 °C, and are rounded with white margins at the circumference. Aerial hypha are cottony to sparse, dark green to greenish brown, and reverse center dark green to pale gray. Sporulation is abundant, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

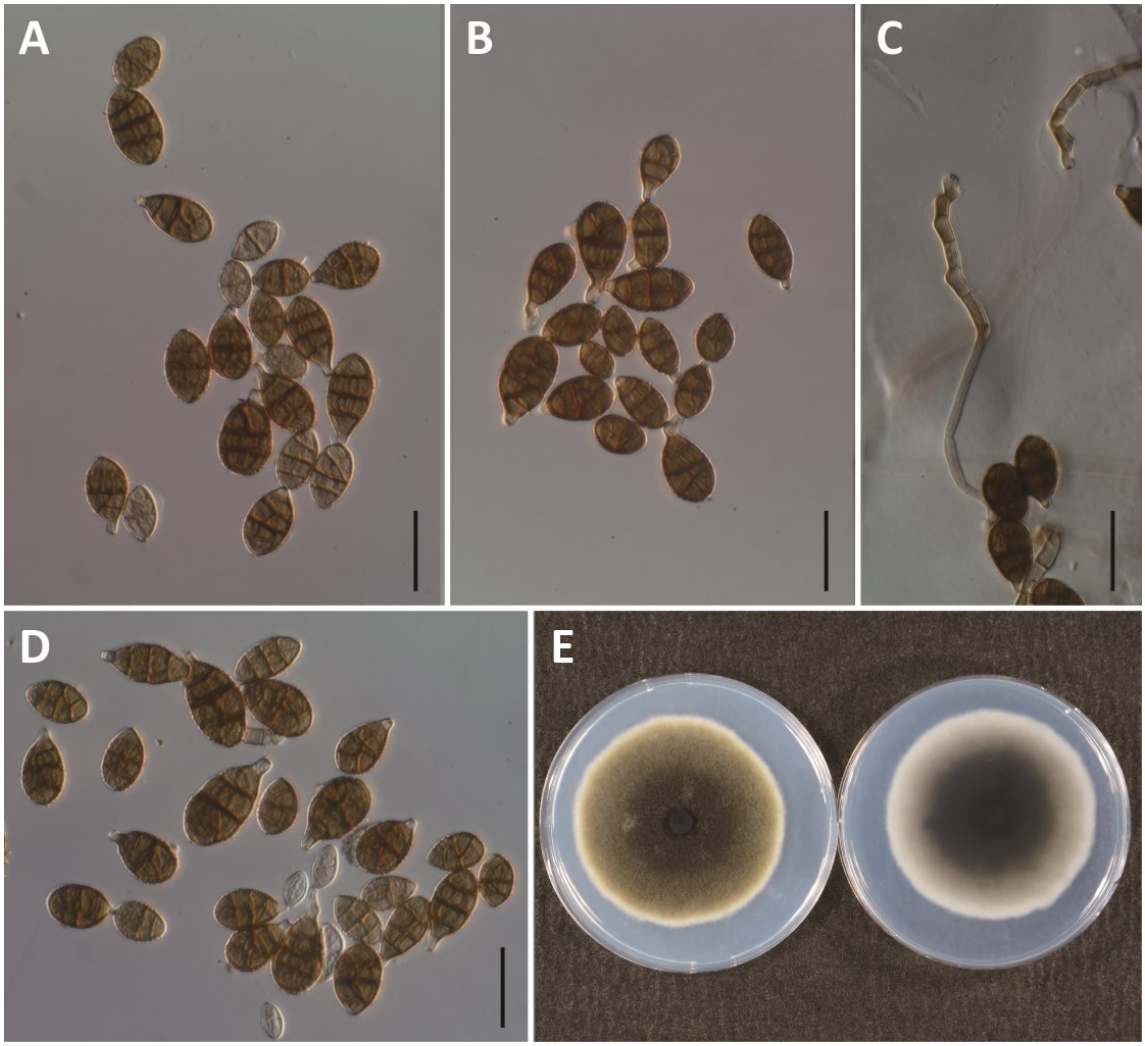

Fig. 5.39. Morphological features of Japanese isolates of *Alternaria chartarum* (AC85). **A–D.** Conidia and conidiophores on potato-carrot agar medium. **E.** Culture on potato-dextrose agar medium (left = surface, right = reverse). Bars (A–D) = 25 µm.

Natural host: Saprophytic, but there are records of pathogenicity to *Quercus pubescens*, *Vaccinium corymbosum*, and *Lippia citriodora* (Vannini & Vettraino 2000; Starast *et al.* 2009; Zarandi & Sharzei 2015).

Distribution: Worldwide, including Asia (Japan, India, Iran, etc.), Europe (Poland, Russia, UK, etc.), North and Latin America (Canada, USA, Uruguay, Nicaragua), and Australia (Rao 1969; Ellis 1976; Phillips *et al.* 1979; Rossman & Lu 1980; Watanabe *et al.* 1986; Richardson 1990; Bettucci *et al.* 1997; Chen *et al.* 2002; Nishikawa *et al.* 2006; Delgado 2011; Kowalski & Andruch 2012; Woudenberg *et al.* 2013; Zarandi & Sharzei 2015; Barkat *et al.* 2016; Farr & Rossman 2018).

Distinctive features: Short conidiophores are geniculate and proliferate frequently; obclavate conidia commonly in appear in chains, with three transverse septa.

Notes: This species has often been misidentified as *A*. *alternata* owing to its conidial catenation (de Hoog & Horré 2002).

Section *Radicina* D.P. Lawr., Gannibal, Peever & B.M. Pryor, *Mycologia* **105**: 541. 2013. [MB 802310].

Five species are recognized in this section (Lawrence *et al.* 2013; Woudenberg *et al.* 2013), which is morphologically characterized by medium-sized, spored, beakless conidia. All recognized species in this section were sourced from *Apiaceae*. Two species, *A*. *petroselini* and *A*. *radicina*, are distributed in Japan (Yoshii 1929b; Nishikawa & Nakashima 2013), and the former was examined in the present study.

Alternaria petroselini (Neerg.) E.G. Simmons, in Ellis, *More dematiaceous Hyphomycetes* (*Kew*): 417. 1976. [MB 308516]. **Figs** 4.6; 4.7a; **5.40**.

≡ *Stemphylium petroselini* Neerg., *Zentralbl. Bakteriol., 2. Abt.* **104**: 411. 1942. [MB 291187].

≡ *Stemphylium radicinum* var. *petroselini* (Neerg.) Neerg., *Danish species of* Alternaria *&* Stemphylium: 357. 1945. [MB 346437].

≡ *Alternaria radicina* var. *petroselini* (Neerg.) Neerg., *Encycl. Mycol.* **33**: 123. 1964. [MB 445538].

= *Macrosporium cheiranthi* f. *petroselini* Sacc., *Rev. Mycol.* (*Toulouse*) **19**: 54. 1897. [MB 148232].

= *Alternaria selini* E.G. Simmons, *Mycotaxon* **55**: 109. 1995. [MB 412387].

Type: **Denmark**, from seeds of *Petroselinum crispum* (Mill.) Fuss, 4 Apr. 1941, *P. Neergaard*, holotype EGS11.062 in **CP** [MBT 128579], culture presumably ex-holotype CBS 112.41 = EGS 06.196.

Collection examined: **Japan**, Shizuoka Prefecture, Kakegawa, on leaves of *Petroselinum crispum* (Mill.) Fuss, 26 April 2007, *J. Nishikawa*, MUMH 11679, living culture MAFF 243057 = AC42.

Morphological character on PCA medium: Conidia are usually solitary, but sometimes appear in chains of 2–3, without lateral branches. They are dark to yellowish brown, broad ovoid to subsphaeroid, obclavate to long ellipsoid, and are commonly smooth, measuring 15–76 × 8–30 µm (l/w = 1.2–3.9), with 1–10 transverse and 0–11 (commonly 1–2 in each transverse segment) longitudinal septa. They are usually beakless, but sometimes have a false beak (secondary conidiophore) at the apex. Conidiophores are geniculate, proliferate sympodially, are short to moderately long, narrow, and occasionally branched, measuring 10–88 × 5–6 µm. Morphology of lesions and when grown on V8 media was similar to those observed when grown on PCA medium.

Culture characteristics on PDA medium: Colonies are fast-growing, reaching 82.1 ± 1.8 mm in diam after 7 d at 25 °C, and are rounded to sometimes irregular at the circumference. Aerial hypha are cottony and dense, gray to dark green, and reverse center dark green to black. Sporulation is abundant, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

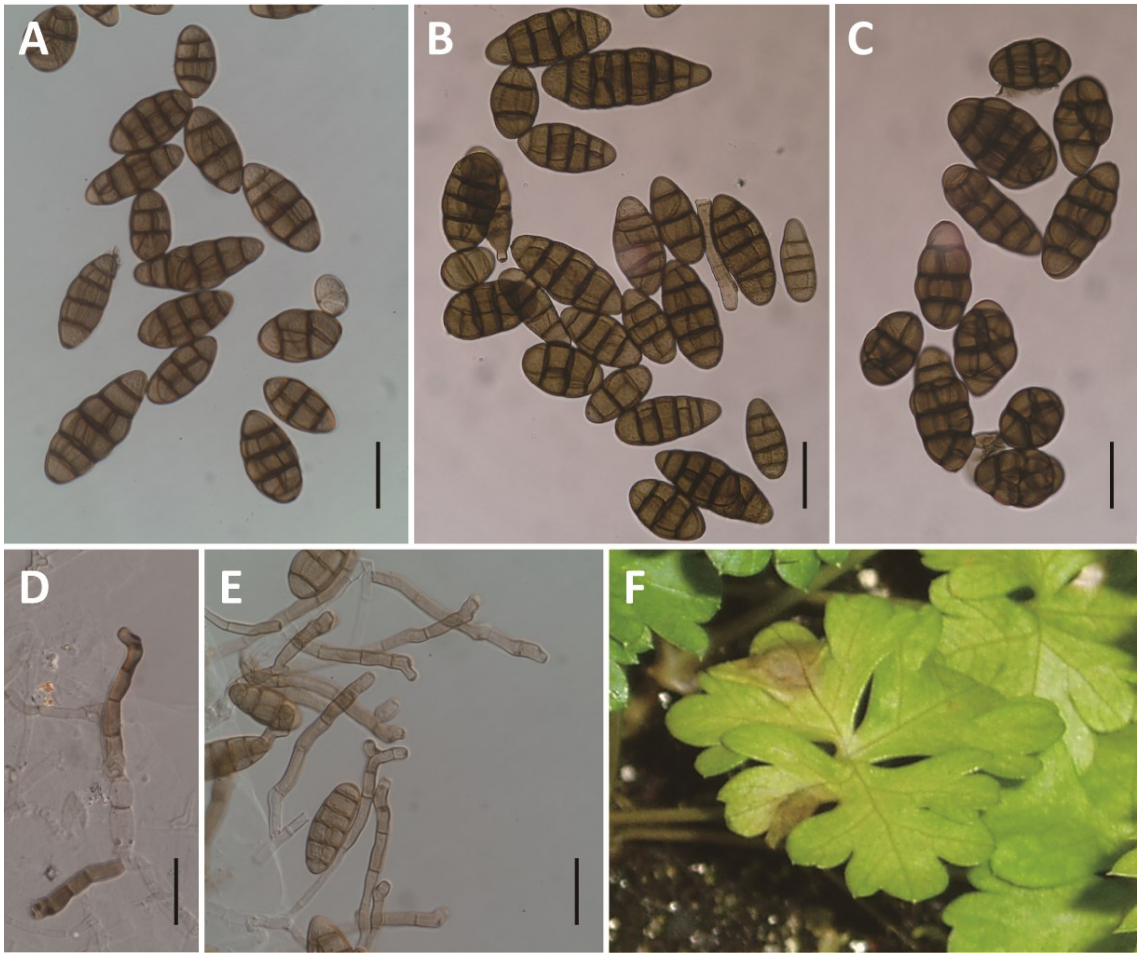

Fig. 5.40. Morphological features of Japanese isolates of *Alternaria petroselini* (MAFF 243057). **A–C.** Conidia on potato-carrot agar (PCA) medium. **D, E.** Conidiophores on PCA medium. **F.** Natural symptoms. Bars (A–E) = 25 µm.

Natural host: Typically *Petroselinum*, *Coriandrum*, and *Foeniculum* (*Apiaceae*), but may also occasionally infect *Carya* (*Juglandaceae*) (Liu *et al.* 2013).

Symptoms: Leaf spots on *Petroselinum* are indistinct, sooty brown, water-soaked, and expand to produce leaf blight.

Experimental host range: Widely pathogenic within *Apiaceae* plants, including *Ammi*, *Anethum*, *Angelica*, *Anthriscus*, *Apium*, *Bupleurum*, and *Cuminum* (Table 4.1).

Distribution: Japan, China, Saudi Arabia, USA, Italy, Netherlands, Spain, UK, and Australia (Ellis 1976; Farrar *et al.* 2004; Cunnington *et al.* 2007; Pryor & Asma 2007; Park *et al.* 2008; Infantino *et al.* 2009; Bassimba *et al.* 2012; Liu *et al.* 2013; Nishikawa & Nakashima 2013; Farr & Rossman 2018).

Distinctive features: Conidia are solitary or appear in a short chains, are mostly beakless, and broad-ovoid to long-ellipsoid (but variable in shape and size). This species was widely pathogenic to *Apiaceae*, but not to *Daucus*, and is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Notes: All the sequences of the examined Japanese isolate MAFF 243057 were strictly identical with those of the ex-type isolates, *A. petroselini* (CBS 112.41) and *A. selini* (CBS 109382). Based on both phylogenetic analysis and morphological observations, *A. selini* was never distinguishable from *A. petroselini* (Fig. 5.2), and, thus, they were synonymized in the present study.

Section *Sonchi* D.P. Lawr., Gannibal, Peever & B.M. Pryor, *Mycologia* **105**: 542. 2013. [MB 802311].

This section is morphologically characterized by large conidia, which are solitary or may appear in short chains with a blunt-tapered false beak. There are only two species assigned to this section (Woudenberg *et al.* 2013), although Lawrence *et al.* (2013) included *A. brassicae* within this section.

Alternaria cinerariae Hori & Enjoji, in Enjoji, *J. Pl. Protect.* (*Tokyo*) **18** (8): 432. 1931. [MB 251428]. **Figs** 2.1; 2.2; **5.41**.

= *Alternaria senecionis* Neerg., *Danish species of* Alternaria *and* Stemphylium: 201. 1945. [MB 284039].

Type: **Japan**, Chiba Prefecture, Chiba, Chiba Prefect. Agric. Exp. Station, on leaves of *Pericallis cruenta* (Masson ex L'Hér.) Bolle, 30 Mar. 1931, and in Apr. to May 1931, *S. Enjoji* (holotype not specified; not preserved).

Lectotype: **Japan**, Chiba Prefect. Agric. Exp. Station, on *Senecio cineraria* DC., 28 Apr. 1931, *S. Enjoji*, in **FU** [MBT 119868] (designated in Simmons 1997).

Epitype (**designated here**): **Japan**, Chiba Prefecture, Narita, on leaves of *Pericallis cruenta* (Masson ex L'Hér.) Bolle, 25 Oct. 2002, *J. Nishikawa*, TNS-F-85448 (dried culture specimen ex MAFF 243059) [MBT 385024], isoepitype MUMH 11691, culture ex-epitype MAFF 243059 = MUCC 1701 = AC3.

Collections examined: **Japan**, Chiba Prefecture, Narita, on leaves of *Pericallis cruenta* (Masson ex L'Hér.) Bolle, 25 Oct. 2002, *J. Nishikawa*, TNS-F-85448 (**epitype**), MUMH 11691 (**isoepitype**), living culture MAFF 243059 = MUCC 1701 = AC3; Ibaraki Prefecture, Tsukuba, Kannondai, on leaves of *Farfugium japonicum* (L.) Kitam., Nov. 2008, *Y. Otani*, MUMH 11694, living culture MAFF 241266 = MUCC 1613 = AC57; ibid., on leaves of *Gynura bicolor* (Willd.) DC., Nov. 2008, *Y. Otani*, MUMH 11695 living culture MAFF 241267 = MUCC 1614 = AC58; Kanagawa Prefecture, Atsugi, on leaves of *Jacobaea maritima* (L.) Pelser & Meijden, 23 Aug. 2017, *Y. Makizumi*, living culture AC138.

Morphological character on V8 medium: Conidia are solitary or may appear in chains of 2–5(–9), rarely with lateral branches. They are faintly yellowish-tan to pale brown, smooth, long ellipsoid to obclavate with a blunt, tapered false beak, mostly straight and laterally symmetrical, ranging from 18–319 µm in total length, and constricted at each transverse septa. Conidial

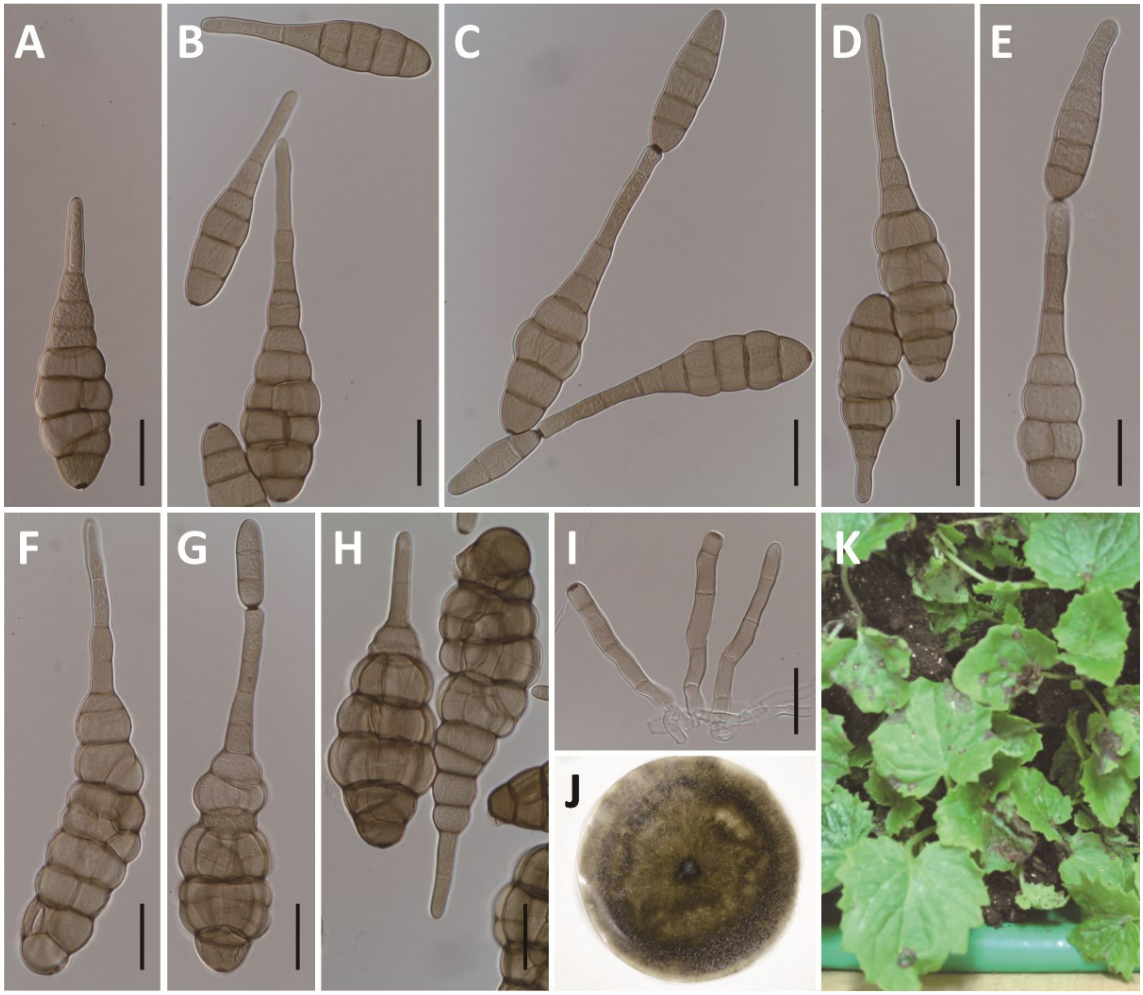

Fig. 5.41. Morphological features of Japanese isolates of *Alternaria cinerariae* (MAFF 243059) on V8 juice agar medium. **A–H.** Conidia. **I.** Conidiophores. **J.** Dried culture specimen ex MAFF 243059 (epitype: TNS-F-85448). **K.** Natural symptoms on *Pericallis cruenta*. Bars (A–I) = 25 µm.

bodies are sometimes excessively swollen, ranging from 18–295 × 8–63 µm, with 1–14 transverse septa and up to 10 longitudinal septa; false beaks unbranched, ranging up to 80–159 × 5–9 µm, concolorous with body, having an inconspicuous border with the conidial body. Conidiophores are broad, ranging from 25–196 × 6–11 µm, and are often branched but are sometimes unbranched. Conidia of ex-epitype culture MAFF 243059 grown on V8 medium are

solitary or appear in chains of 2–3 conidia. Conidial bodies measure 30–138 × 9–46 µm, with 2–12 transverse and up to 10 longitudinal septa. Secondary conidiophores measure up to 123 × 5–9 µm.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching an average of 62.9 ± 3.6 mm in diam after 7 d at 25 °C, though there are variations among strains, and are rounded with white margins at the circumference. Aerial hypha are cottony, grayish green to dark green, and reverse center black to dark green. Sporulation is sparse, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Farfugium*, *Gynura*, *Jacobaea*, *Ligularia*, *Pericallis*, and *Senecio* (*Asteraceae*).

Symptoms: Leaf spots on *Pericallis* are black, circular to irregular, and measure 3–10 mm in diam, often with a necrotic eye at the center. They appear water-soaked, enlarge, and become confluent.

Experimental host range: Selectively pathogenic to tribe *Senecioneae*, and experiments suggest weak pathogenicity to *Cosmos bipinnatus* and *Centaurea* (Table 2.1).

Distribution: Worldwide, but few records exist; Japan, Korea, Denmark, UK, Germany, USA, South Africa, and New Zealand (Enjoji 1931; Neergaard 1945; Ellis 1976; Richardson 1990; Yu 2001; Simmons 2007; Woudenberg *et al.* 2013; Nishikawa & Nakashima 2015; Farr & Rossman 2018).

Distinctive features: Conidia are large, solitary or in short chains with a blunt-tapered false beak. Conidiophores are long, broad, and sometimes branching. This species is selectively pathogenic to tribe *Senecioneae*, which includes genera *Senecio*, *Farfugium* and *Gynura*, and is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Notes: Morphological variations are present between strains, including the appearance of excessively swollen bodies and chlamydospore (microsclerotia) formation (Nishikawa & Nakashima 2015). There is no ex-type culture and few reference isolates, and the epitype originated near the original type locality; therefore, an ex-epitype isolate was designated and deposited for further studies.

Section *Ulocladioides* Woudenb. & Crous, *Stud. Mycol.* **75**: 204. 2013. [MB 803746].

There are ten species and a representative strain of *A. botrytis* assigned to this section (Woudenberg *et al.* 2013), which is typified by *A. cucurbitae*, and consists of a majority of the former *Ulocladium* spp.

Alternaria atra (Preuss) Woudenb. & Crous, *Stud. Mycol.* **75**: 204. 2013. [MB 803717]. **Fig. 5.42**.

≡ *Ulocladium atrum* Preuss, *Linnaea* **25**: 75. 1852. [MB 163775].

≡ *Stemphylium atrum* (Preuss) Sacc., *Syll. Fung.* **4**: 520. 1886. [MB 218045].

= *Alternaria abietis* Tengwall, *Meded. Phytopath. Lab.* '*WCS*' **6**: 50. 1924. [MB 255562].

Type: **Germany**, Hoyerswerda, on *Betula pubescens* Ehrh. (as *Betula alba* L.), *Preuss*, in **B**.

Epitype: **USA**, California, from soil, Nov. 1962, *P.M.D. Martin*, BPI 444871 [MBT 14348] (designated in de Hoog & Horré 2002), culture ex-epitype CBS 195.67 = ATCC 18040 = IMI 124944 = QM 8408.

Collections examined: **Japan**, Shizuoka Prefecture, Kakegawa, on *Allium fistulosum* L., 6 Apr. 2006, *J. Nishikawa*, living culture AC39; Tokyo, Setagaya, from seeds of *Raphanus sativus* L., Jul. 2000, *J. Nishikawa*, living culture AC86; ibid., from seeds of *Brassica oleracea* var. *capitata* L., 4 Feb. 2001, *J. Nishikawa*, living culture AC87; ibid., from seeds of *Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt, 18 Mar. 2001, *J. Nishikawa*, living culture AC88; ibid., from seeds of *B.*

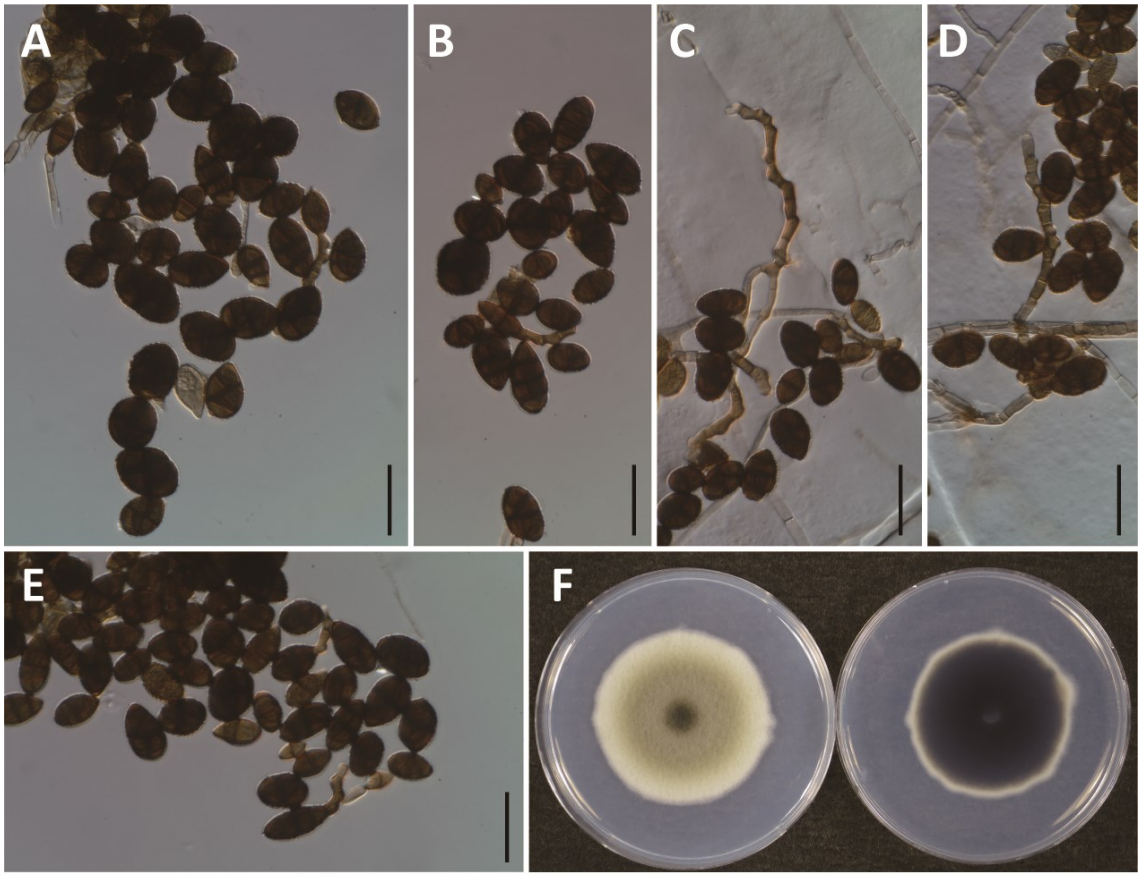

Fig. 5.42. Morphological features of Japanese isolates of *Alternaria atra* (AC90). **A–E.** Conidia and conidiophores on potato-carrot agar medium. **F.** Culture on potato-dextrose agar medium (left = surface, right = reverse). Bars (A–E) = 25 µm.

rapa, 15 Oct. 2001, *J. Nishikawa*, living culture AC89; ibid., from seeds of *Allium fistulosum* L., 7 Jul. 2001, *J. Nishikawa*, living culture MAFF 246889 = AC90.

Morphological character on PCA medium: Conidiophores are solitary, usually unbranched and geniculate, frequently proliferating sympodially. They are pale brown to brown, measuring 23–73 × 3–5 µm, with pores for polytretic sporulation. Conidia are commonly solitary, subsphaeroid to obovoid, brown to dark brown, roughened to conspicuously verrucose, and

measure 10–33 × 6–17 µm, with 0–3 (mostly 1) transverse and 0–2 longitudinal septa. They are usually beakless, but sometimes they have a secondary conidiophore at the apex. Secondary conidiophores also are geniculate, measuring 3–38 × 3–5 µm.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching an average of 65.3 ± 3.1 mm in diam after 7 d at 25 °C, though there are variations between strains, and are rounded with white margins at the circumference. Aerial hypha are cottony to sparse, grayish green to dark green or black, and reverse center pale gray to dark green or black. Sporulation is commonly abundant, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: Saprophytic, but a few records suggest pathogenicity to *Helianthus annuus* and *Solanum tuberosum* (Shtienberg 1994; Esfahani 2018).

Distribution: Worldwide, including Asia (Japan, Israel, Saudi Arabia, Iran, etc.), Europe (Germany, Italy, UK, etc.), North and Latin America (USA, Mexico, Canada, Argentina), Africa (Egypt, etc.), and the Pacific (Australia and New Zealand) (Ellis 1976; Abdel-Hafez 1984; Shtienberg 1994; Heredia *et al.* 1995; Chen *et al.* 2002; Lunghini *et al.* 2013; Esfahani 2018; Farr & Rossman 2018).

Distinctive features: Morphological characteristics of the former genus *Ulocladium*; conidiophores are geniculate and frequently proliferate; conidia are sometimes solitary, but often appear in chains with secondary conidiophores, and are obovoid, generally with one transverse septum.

Section *Ulocladium* (Preuss) Woudenb. & Crous, *Stud. Mycol.* **75**: 206. 2013. [MB 803747].
≡ *Ulocladium* Preuss, *Linnaea* **24**: 111. 1851. [MB 10346].

Four species typified by *A*. *botrytis* were assigned to this section by Woudenberg *et al*. (2013), and former *Sinomyces* spp., which is *S*. *alternariae*, *S*. *fusoideus* and *S*. *obovoideus*, may be also included in this section. Two species, *A*. *botrytis* and *A*. *oudemansii*, are found in Japan (Katumoto 2010), though there are few morphological differences between the species in this section (Simmons 1967; Runa *et al.* 2009).

Alternaria botrytis (Preuss) Woudenb. & Crous, *Stud. Mycol.* **75**: 206. 2013. [MB 803718]. **Fig. 5.43**.

≡ *Ulocladium botrytis* Preuss, *Linnaea* **24**: 111. 1851. [MB 163248].

≡ *Stemphylium botryosum* Wallr. var. *ulocladium* Sacc., *Syll*. *Fung*. **4**: 522. 1886. [MB 138358].

≡ *Stemphylium botryosum* Wallr. var. *botrytis* (Preuss) Lindau, *Rabenh*. *Krypt*.-*Fl*., Edn 2 **1**(9): 219. 1908. [MB 508271].

Type: **Germany**, Hoyerswerda, on wood sliver of *Quercus*, in **B**.

Epitype: **USA**, Cambridge, Massachusetts, contaminant (air), CBS H-19057 (designated in de Hoog & Horré 2002) [MBT 107672], culture ex-epitype CBS 197.67 = ATCC 18042 = IMI 124942 = MUCL 18556 = QM 7878.

Collection examined: **Japan**, Shizuoka Prefecture, Kakegawa, from rhizomes of *Asparagus officinalis* L., 8 Apr. 2008, *J. Nishikawa*, living culture MAFF 246887 = AC52.

Morphological character on PCA medium: Conidiophores are solitary, often branched, geniculate, and frequently proliferate sympodially. They are pale brown to brown, measuring 48–145 × 2–4 μm, with pores for polytretic sporulation. Conidia are commonly solitary, brown to dark brown, roughened to conspicuously verrucose, obovoid to ellipsoid, and beakless, measuring 13–30 × 8–17 μm, with 1–3 (mostly 3) transverse and 0–3 longitudinal septa.

Culture characteristics on PDA medium: Colonies are moderate-growing, reaching 68.1 ± 0.9 mm in diam after 7 d at 25 °C, and are rounded with white margins at the circumference. Aerial

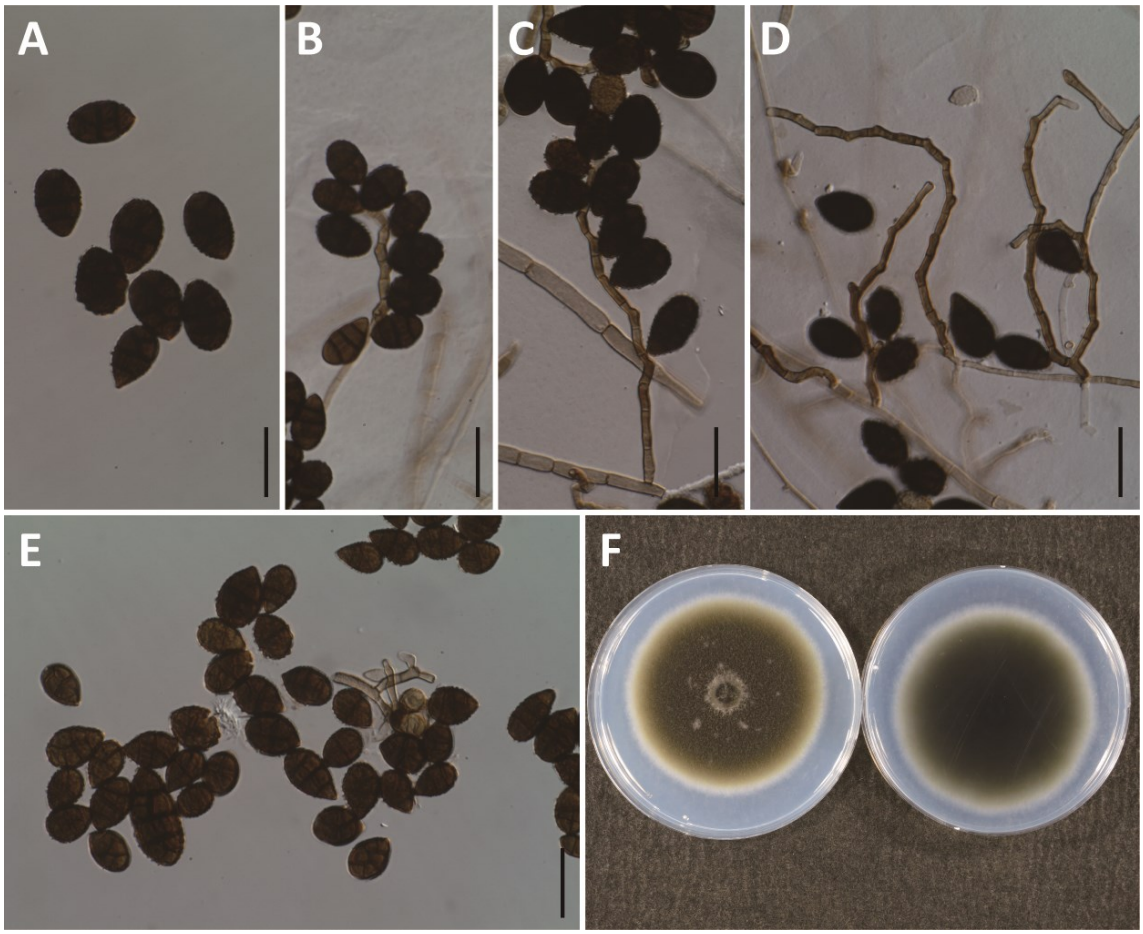

Fig. 5.43. Morphological features of Japanese isolates of *Alternaria botrytis* (MAFF 246887). **A–E.** Conidia and conidiophores on potato-carrot agar medium. **F.** Culture on potato-dextrose agar medium (left = surface, right = reverse). Bars (A–E) = 25 µm.

hypha are commonly sparse, green to greenish brown, and reverse center black to dark green. Sporulation is abundant, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: saprophytic (recorded on *Pinus*, *Alnus, Betula*, etc., but no records exist suggesting pathogenicity).

Distribution: Japan, Thailand, China, India, Kuwait, Pakistan, USA, Uruguay, Scotland, Poland, Russia, Germany, and Egypt (Ellis 1971; Tokumasu *et al.* 1994; Alonso *et al.* 2011; Farr & Rossman 2018).

Distinctive features: Morphological characteristics of the former genus *Ulocladium*; conidiophores are geniculate and frequently proliferate; conidia are solitary and typically obovoid, usually with three transverse septa.

Notes: Phylogenetic analysis conducted during the present study, as well as morphological similarity, suggest that this species is conspecific with *A*. *alternariae* and *A*. *oudemansii* (Runa *et al.* 2009; Woudenberg *et al.* 2013) (Fig. 5.2), which have already been observed on pine and Japanese cedar seeds (Wicker & Yokota 1982; Watanabe *et al.* 1986, Watanabe & Sato 1988).

Monotypic lineages

Woudenberg *et al.* (2013) recognized six species as single species not assigned to hitherto known sections, namely *Alternaria argyranthemi* E.G. Simmons & C.F. Hill, *A*. *brassicae*, *Alternaria dennisii* M.B. Ellis, *Alternaria helianthiinficiens* E.G. Simmons, Walcz & R.G. Roberts, *Alternaria soliaridae* E.G. Simmons, and *Alternaria thalictrigena* K. Schub. & Crous. Lawrence *et al.* (2016) recognized an additional two monotypic lineages, *Alternaria peucedani* S.H. Yu and *Alternaria thlaspis* (E.G. Simmons & J.C. David) D.P. Lawr., Rotondo & Gannibal. Among these, *A*. *brassicae* is already known in Japan, and a novel species isolated from *Bupleurum* were also newly described here as a ninth monotypic lineage.

Alternaria brassicae (Berk.) Sacc., *Michelia* **2**: 172. 1880. [MB 214057]. **Figs 5.44**, **45**.

≡ *Alternaria brassicae* f. *brassicae* (Berk.) Sacc., *Michelia* **2**: 172. 1880. [MB 419459].

≡ *Alternaria brassicae* subsp. *brassicae* (Berk.) Sacc., *Michelia* **2**: 172. 1880. [MB 568543].

≡ *Alternaria brassicae* var. *brassicae* (Berk.) Sacc., *Michelia* **2**: 172. 1880. [MB 419467].

≡ *Macrosporium brassicae* Berk., in Smith, *Engl. Fl., Fungi* **5** (2): 339. 1836. [MB 237664].

≡ *Macrosporium brassicae* var. *brassicae* Berk., in Smith, *Engl. Fl., Fungi* **5** (2): 339. 1836.

≡ *Macrosporium brassicae* f. *brassicae* Berk., in Smith, *Engl. Fl., Fungi* **5** (2): 339. 1836.

= *Puccinia brassicae* Mont., *Ann. Sci. Nat., Bot.* **6**: 30. 1836. [MB 234279].

≡ *Rhopalidium brassicae* (Mont.) Mont., *Ann*. *Sci*. *Nat*., *Bot*. **6**: 30. 1836. [MB 201138].

≡ *Dicaeoma brassicae* (Mont.) Kuntze, *Revis. Gen. Pl.* **3** (2): 468. 1898. [MB 525194].

= *Sporidesmium exitiosum* J.G. Kühn, *Hedwigia* **1**: 91. 1855. [MB 101874].

= *Sporidesmium exitiosum* var. *exitiosum* J.G. Kühn, *Hedwigia* **1**: 91. 1855.

= *Sporidesmium exitiosum* f. *exitiosum* J.G. Kühn, *Hedwigia* **1**: 91. 1855.

≡ *Polydesmus exitiosus* (J.G. Kühn) Rabenh., *Klotzsch*. *Herb*. *Vivum Mycol*. *Sistems Fungorum German*., *Ed*. *Nov*.: no. 181. 1855.

≡ *Polydesmus exitiosus* (J.G. Kühn) J.G. Kühn, *Krankh*. *Kulturgew*.: 165. 1859, nom. illeg. [comb. superfl., later isonym of *P. exitiosus* (J.G. Kühn) Rabenh.; Art. 52.1]; [MB 212109].

≡ *Polydesmus exitiosus* f. *exitiosus* (J.G. Kühn) J.G. Kühn, *Krankh*. *Kulturgew*.: 165. 1859. [MB 485731].

≡ *Polydesmus exitiosus* var. *exitiosus* (J.G. Kühn) J.G. Kühn, *Krankh*. *Kulturgew*.: 165. 1859. [MB 419969].

≡ *Alternaria brassicae* (Berk.) Sacc. var. *exitiosa* (J.G. Kühn) Ferraris, *Fl*. *Ital*. *Crypt*. **1** (8): 521. 1912. [MB 455464].

≡ *Alternaria exitiosa* (J.G. Kühn) Jørst., *Meld. Stat. Plantepatol. Inst. Oslo* **50**: 94. 1945. [MB 284027].

= *Macrosporium herculeum* Ellis & G. Martin, *Amer. Naturalist* **16**: 1003. 1882. [MB 207895].

≡ *Alternaria herculea* (Ellis & G. Martin) J.A. Elliott, *Am. J. Bot.* **4**: 472. 1917. [MB 102341].

= *Cercospora bloxamii* Berk. & Broome, *Ann. Mag. Nat. Hist*. **9**: 183. 1882. [MB 159802].

= *Cercospora lepidii* Peck, *Rep.* (*Annual*) *New York State Mus. Nat. Hist.* **35**: 140. 1884. [MB 248041].

= *Alternaria brassicae* (Berk.) Sacc. var. *macrospora* Sacc., *Syll. fung.* **4**: 546. 1886. [MB

137505].

≡ *Alternaria macrospora* (Sacc.) Mussat, in Saccardo, *Syll. fung.* **15**: 43. 1900, nom. inval. (Art. 34.1). [MB 206538].

≡ *Alternaria macrospora* (Sacc.) Sawada, *Rep. Dept. Agric. Gov. Res. Inst. Formosa* **51**: 123. 1931. [MB 505858].

≡ *Alternaria saccardoi* Sawada, *Spec. Publ. Natl. Taiwan Univ. Coll. Agric.* **8**: 208. 1959. [MB 326072].

= *Sporidesmium onnii* P. Karst., *Symb*. *Mycol*. *Fenn*. **30**: 67. 1891. [MB 162519].

= *Macrosporium brassicae* var. *macrosporum* A.G. Eliasson, *Bih. Kongl. Svenska Vetensk.-Akad. Handl.* **22** (12): 18. 1897. [MB 139023].

≡ *Macrosporium macrosporum* (A.G. Eliasson) Sawada, *Bull*. *Gov*. *Forest Exp*. *Sta*. **105**: 101, 1958. [MB 317104].

= *Sporidesmium brassicae* Massee, *Bull*. *Misc*. *Inform*. *Kew*: 153, 1901. [MB 198867].

≡ *Alternaria brassicae* (Berk.) P.C. Bolle, *Meded*. *Phytopathol*. *Lab*. "*Willie Commelin Scholten*" **7**: 27. 1924.

= *Alternaria alliariae-officinalis* Săvul. & Sandu, *Hedwigia* **73**: 130. 1933. [MB 256406].

= *Cercospora moldavica* Săvul. & Bontea, in Săvulescu, *Herb*. *Mycol*. *Roman*., *Fasc*. **27**: no. 1336. 1947. [MB 294479].

Type: **UK**, Northamptonshire, Kings Cliffe, on decaying leaves of *Brassica oleracea* var. *capitata* L., *M.J. Berkeley* (holotype specimen unknown according to Simmons 1995a).

Neotype: **UK**, Essex, on leaves of *B. oleracea* var. *capitata*, 16 Oct. 1966, *E.G. Simmons*, IMI 369156 (designated in Simmons 1995a) [MBT 121030 / MBT 52415].

Ex-type culture: Unknown.

Collections examined: **Japan**, Shizuoka Prefecture, Kakegawa, from seeds of *Brassica rapa* L., 15 Aug. 2006, *J. Nishikawa*, living culture AC29; Ibaraki Prefecture, Tsukuba, on leaves of *Raphanus sativus* L., Jul. 2007, *T. Sato*, living culture MAFF 240791; Chiba Prefecture, Narita,

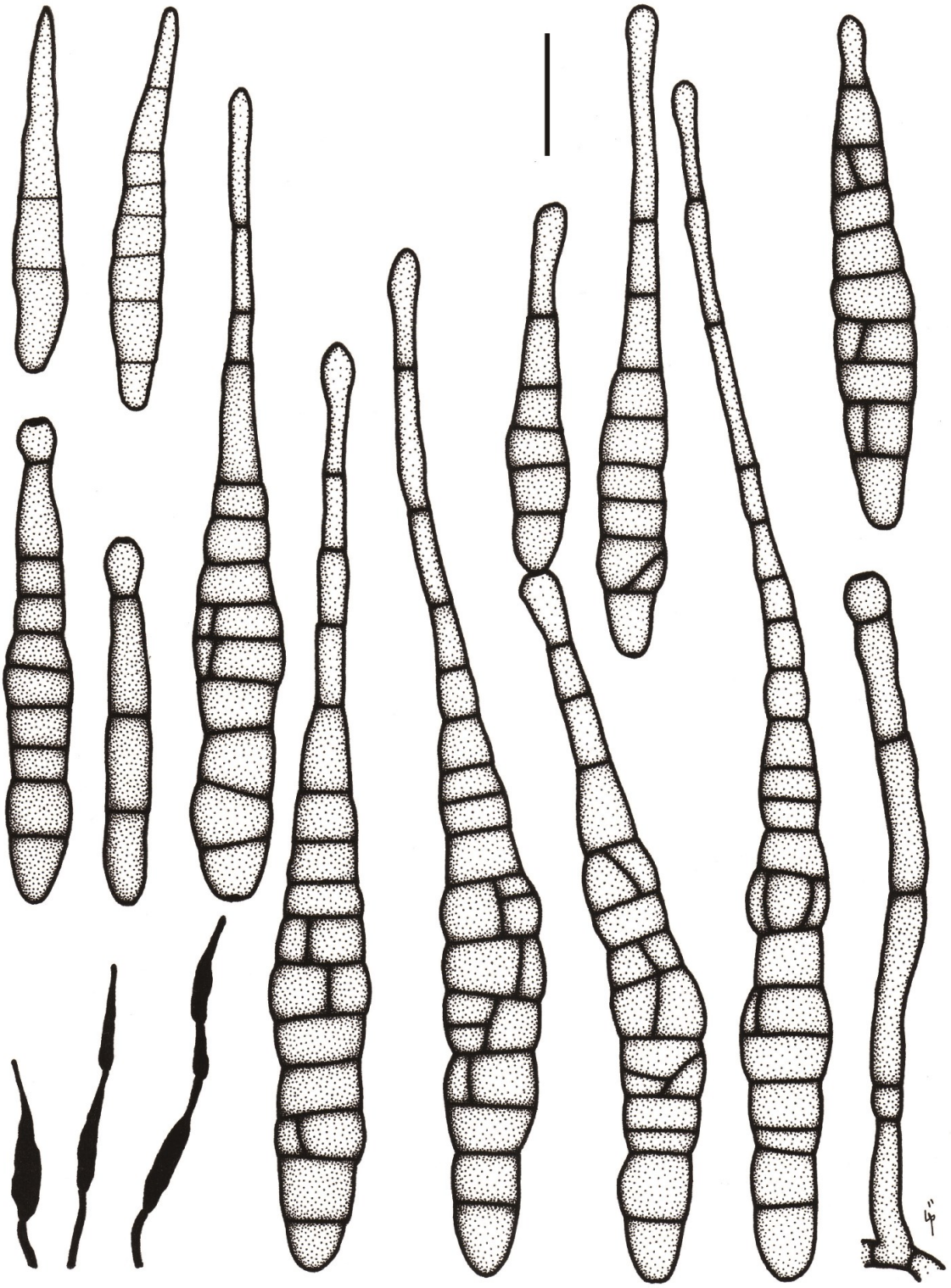

Fig. 5.44. Illustrations of *Alternaria brassicae* (MAFF 240791). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on V8 juice agar medium. Bar = 25 µm.

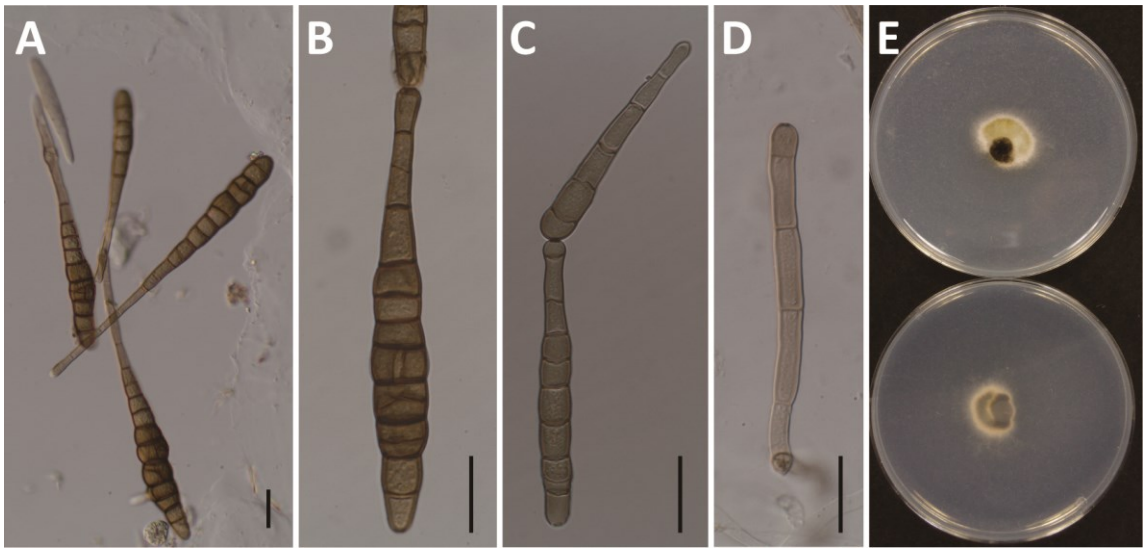

Fig. 5.45. Morphological features of Japanese isolates of *Alternaria brassicae* (MAFF 240791) on V8 juice agar medium. **A–C.** Conidia. **D.** Conidiophore. **E.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). Bars (A–D) = 25 µm.

Minami-misatozuka, on leaves of *R. sativus*, 13 Nov. 2009, *J. Nishikawa*, MUCC 11684, living culture MUCC 1615 = AC62.

Morphological character on V8 medium: Conidia are solitary or appear in chains of 1–2, are pale brown to brown, subcylindrical to oblong, with blunt-tapered beaks, ranging from 40–237 µm in total length. Conidial bodies range from 33–160 × 8–33 µm, with 1–10 transverse and 0–9 longitudinal septa, and their surface is commonly smooth. Beaks are straight, not filamentous, unbranched, and concolorous with the bodies, measuring 6–121 × 3–10 µm. Conidiophores are pale brown to brown and broad, measuring 38–183 × 6–11 µm.

Culture characteristics on PDA medium: Colonies are slow-growing, reaching an average of 37.6 ± 1.6 mm in diam after 7 d at 25 °C, and are rounded at the circumference. Aerial hypha

are cottony, white to pale gray, and reverse center black to dark green. Sporulation is sparse, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

Natural host: *Brassicaceae* (*Arabis*, *Armoracia*, *Brassica*, *Bunias*, *Camelina*, *Cochlearia*, *Crambe*, *Descurainia*, *Eruca*, *Eutrema*, *Iberis*, *Lepidium*, *Lunaria*, *Neslia*, *Radicula*, *Raphanus*, *Rorippa, Sinapis*, *Sisymbrium*, and *Sisymbrium*), *Cucumis sativa* and *Cucurbita pepo* (*Cucurbitaceae*), and *Beta vulgaris* (*Amaranthaceae*) are correct source plants according to Simmons (2007). All other recorded hosts reported by Farr & Rossman (2018) may be listed under the names of each formae and variety of *A*. *brassicae*.

Symptoms: Small, black spots appear on the leaves and petioles of *Raphanus*. They are 5 mm in diam, circular to zonate, with a necrotic eye at the center, becoming enlarged and confluent.

Experimental host range: Pathogenic to *Brassicaceae*, including *Diplotaxis* (*Brassiceae*), *Eutrema* (*Eutremeae*), *Lobularia* (*Alysseae*), *Matthiola* (*Anchonieae*), *Iberis* (*Iberideae*), and *Nasturtium* (*Cardamineae*), but not to *Aubrieta* (*Arabideae*), *Capsella* (*Lepidieae*), and non-*Brassicaceae* plants (Table 5.5).

Distribution: Worldwide, including Asia (Japan, Korea, Bangladesh, China, India, etc.), Europe (Russia, Serbia, UK, Austria, Denmark, etc.), North and Latin America (USA, Argentina, Haiti, Bolivia, Nicaragua, Canada, etc.), Africa (South Africa, etc.), and the Pacific (Australia, New Zealand, etc.) (Farr & Stevenson 1963; Benjamin & Slot 1969; Rao 1969; Richardson 1990; Jasalavich *et al.* 1995; Koike 1996; Koike & Molinar 1997; Crous *et al.* 2000; Cho *et al.* 2001; Yu 2001; Zhang 2003; Gaetan & Madia 2005; You *et al.* 2005; Simmons 2007; Caesar & Lartey 2009; Gannibal & Gasich 2009; Woudenberg *et al.* 2013; Blagojević *et al.* 2015; van de Wouw *et al.* 2016; Farr & Rossman 2018).

Distinctive features: Large spores with blunt-tapered false beaks, and slow-growing on PDA medium. This species is widely pathogenic to *Brassicaceae*, including *Eutrema*, but not to *Aubrieta* and *Capsella*. It is phylogenetically recognizable via its ITS sequence (Fig. 5.3).

Alternaria triangularis Jun. Nishikawa & C. Nakash., ***sp. nov.*** MycoBank MB 829137. **Figs 5.46**, **47**.

Etymology: Named after Latin "triangularis", referring to the triangular shape of the conidia.

Diagnosis: The morphology of this species includes isosceles triangle-shaped conidia, comprising multi-cell bodies and elongated secondary conidiophores, and is quite unique among *Apiaceae* species. Phylogenetic analysis suggested that this species is a monotypic lineage sister to the sect. *Sonchi* and monotypic lineage *A*. *brassicae*. The species is also characterized by its host range, which is restricted to *Bupleurum rotundifolium*.

Descriptions: Leaf spots are circular, 4–10 mm in diam, dark brown to black with a grayish eye at center, and are distinct at the border; leaf defoliation follows. When grown on V8 medium, conidia appear primarily in chains of 3–5, up to 8–9 (short to moderately long chains), and lateral branches are uncommon 5–7 d after incubation. Conidia are pale brown to brown, long ovoid to obclavate, triangular to campanuloid in maturity, and 15–93 µm in total length. Conidial bodies measure 14–53 × 6–33 µm, with 1–9 transverse septa and 0–11 (commonly present in each unit, and sometimes complexed) longitudinal septa. The basal 1–2 units is the broadest part, and the bottom of the conidium is almost flattened, with a smooth to faintly rough surface. False beaks (secondary conidiophores) are often elongated in 2–3 cells measuring up to 47 × 6 µm. Conidiophores are short to moderately long, narrow, and measure 16–59 × 4–6 µm. Conidia on lesions measure 10–76 µm in total length; conidial bodies measure 10–45 × 5–23 µm, with 0–9 transverse septa and 0–9 longitudinal septa. False beaks measure up to 41 × 6 µm. Conidiophores measure 19–67 × 3–6 µm.

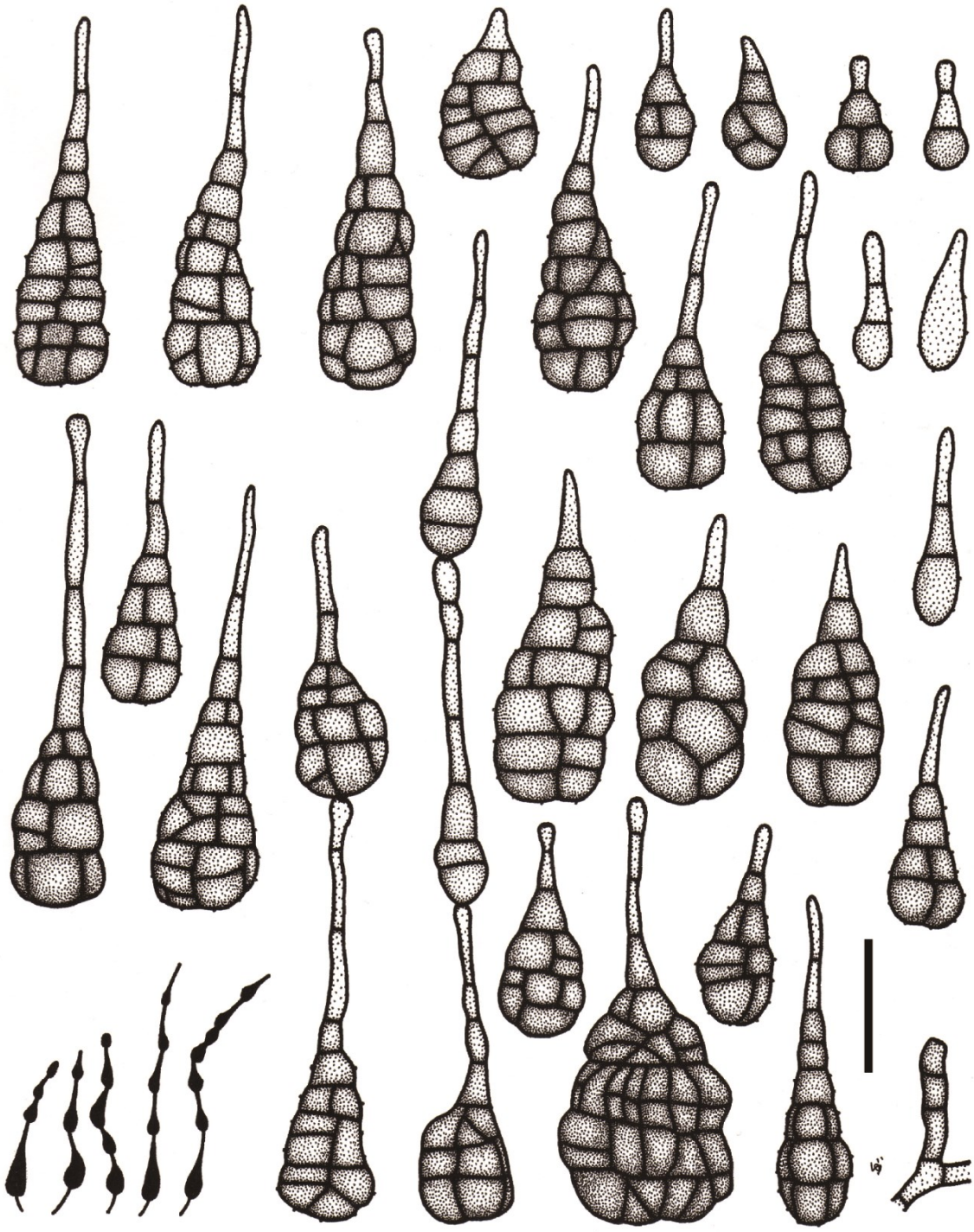

Fig. 5.46. Illustrations of *Alternaria triangularis* (ex-holotype culture MAFF 246776). Morphology of conidia and conidiophores, and sporulation patterns (opaque) on V8 juice agar medium. Bar = 25 µm.

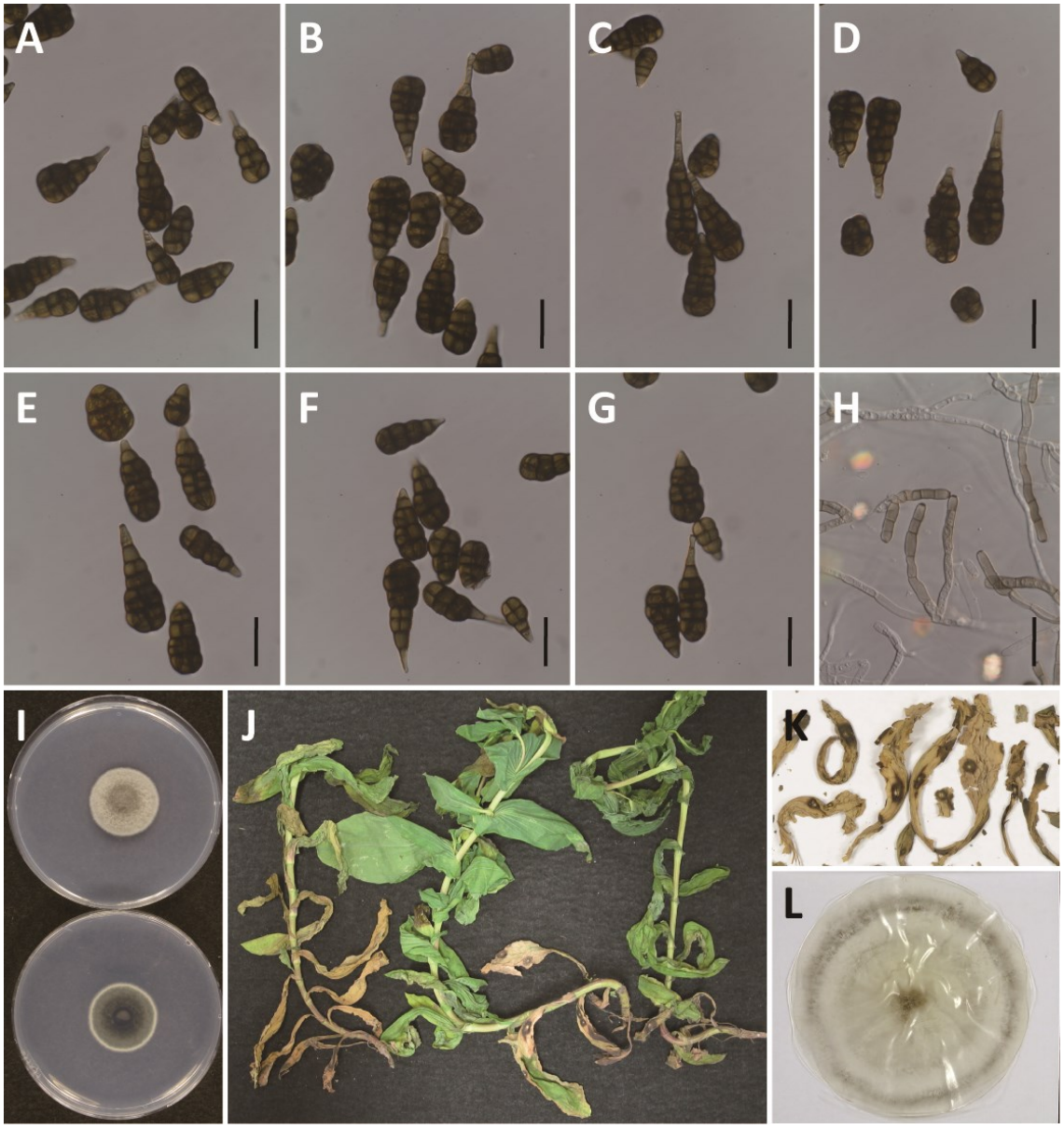

Fig. 5.47. Morphological features of Japanese isolates of *Alternaria triangularis* (ex-holotype culture MAFF 246776). **A–G.** Conidia on V8 juice agar (V8) medium. **H.** Conidiophores on V8 medium. **I.** Culture on potato-dextrose agar medium (upper = surface, lower = reverse). **J–K.** Natural symptoms on *Bupleurum*. **L.** Dried culture specimen ex MAFF 246776 (holotype: TSN-F-85454). Bars (A–H) = 25 µm.

Type: **Japan**, Kochi Prefecture, Konan, on leaves of *Bupleurum rotundifolium* L., 9 Jan. 2004, *J. Nishikawa*, holotype TNS-F-85454 (a dried culture specimen ex MAFF 246776) [MBT 385061], isotype MUMH 11669 and 11700, culture ex-holotype MAFF 246776 = AC5, GenBank accession number ITS: LC440629.

Additional collection examined: **Japan**, Shizuoka Prefecture, Kakegawa, on *Bupleurum rotundifolium* L., 7 Jun. 2004, *Y. Makizumi*, living culture AC95.

Distribution and host range: Only known in Japan. Selectively pathogenic to *Bupleurum* among members of the *Apiaceae* family (Table 5.6).

Culture characteristics on PDA medium: Colonies are slow-growing, reaching an average of 23.9 ± 0.9 mm in diam after 7 d at 25 °C, and are rounded with white margins at the circumference. Aerial hypha are cottony and dense, grayish green to dark green, and reverse center dark green to black. Sporulation is sparse, and no pigment is released into the medium.

Teleomorph: Not observed on RSA medium.

DISCUSSION

During our survey of *Alternaria* species in Japan, we obtained and examined 85 isolates. Based on morphological observations, 23 existing species (including four species newly recorded in Japan) and three novel species were found. Moreover, multi-locus phylogeny and phenotyping with experimental host range not only determined each species boundary, but also revealed closely related and indistinguishable taxa of the examined Japanese species.

In the present study, five Japanese species with two formae speciales were clearly recognized in sect. *Alternaria*. Based on the conidial morphology of *A. iridicola* on holotype

specimens, Simmons (2007) determined the taxonomic affinity of the species with small-spored species, namely within sect. *Alternaria*. Although Gannibal & Lawrence (2018) suggested that those of Russian isolates had intermediate characteristics in both sect. *Panax* and *Porri*, its morphology was nearly identical to ex-type descriptions (Ellis & Everhart 1894; Simmons 2007) and the phylogeny of the Japanese isolates revealed that this species clearly belongs to sect. *Alternaria* (Fig. 5.2). A dried-culture specimen (TNS-F-85452) was deposited as an epitype of *A*. *iridicola*. There are two related taxa, *A*. *iridiaustralis* and *A*. *iridis*, infecting *Iris* spp. within the section. The results of the inoculation test with *A*. *iridicola* demonstrate that this species is not pathogenic to *I*. *ensata* (Table 5.8), which is a natural host of *A*. *iridiaustralis* (Luo *et al.* 2018). Therefore, these related species were distinguishable from *A*. *iridicola* by host selectivity, as well as morphology and phylogeny. In addition, a novel species, *A*. *cylindrica* having selective pathogenicity to *Petunia*, was described, and some of the other species in this section (e.g., *A*. *alstroemeriae* and *A*. *gaisen* in the present study) also have host selectivity within a genus, species, or variety. In this way, species in this section generally have distinct host selectivity, and it was possible that potential host-selective toxin producers may be present.

In sect. *Alternantherae*, *A*. *paragomphrenae* was newly described in the present study. It was suggested that species in sect. *Alternantherae* were clearly differentiated in their pathogenicity to *Amaranthaceae* plants, reflecting their morphological and phylogenetical differences (Table 5.3, 5.4; Fig. 5.4). Two species infecting *Gomphrena*, *A*. *gomphrenae* and *A*. *paragomphrenae*, were non-pathogenic to *Amaranthoideae* plants, including *Amaranthus* and *Celosia*, and were distinguishable from each other in pathogenicity to *Alternanthera* (Table 5.4). The remaining species had respectively wide host ranges across the subfamilies in *Amaranthaceae*, and *A*. *celosiicola* was pathogenic to *Amaranthoideae* and *Gomphrenoideae*. In addition, it was considered that *A*. *alternantherae* and *A*. *perpunctulata* were conspecific, as they share a common original host (*Alternanthera*), are morphologically similar, and show high phylogenetic affinity (Zhao & Zhang 2005).

In sect. *Brassicicola*, Japanese isolates of *A. brassicicola* including ex non-Brassica isolate MAFF 246773 were equally pathogenic to a wide range of *Brassicaceae* hosts (Table 5.5). However, these isolates are clustered into well-supported single lineage together with ex-type isolates of *A. mimicula*, *A. septorioides*, and *A. solidaccana*, which are isolated from non-Brassica hosts—*Solanum*, *Reseda*, and soil, respectively (Fig. 5.2). Based on their host ranges within *Brassicaceae*, morphological similarity, and ubiquitousness of *A. brassicicola* (Simmons 2007; Farr & Rossman 2018), these three names were synonymized, and it was supposed that this section is a possible monotypic lineage.

Likewise in sect. *Japonicae*, Japanese isolates of *A. japonica* were restricted within *Brassicaceae* (Table 5.5), and their conspecificities to *A. nepalensis*, the ex-type of which is isolated from *Brassica* sp., were supported by phylogenetic analyses conducted during the present study (Fig. 5.2). Because of its morphological similarity to the original description of *A. nepalensis* (Simmons 2007), this species was synonymized with *A. japonica*, and this section was typified as a monotypic lineage. It is interesting that during the evolution and differentiation of the genus *Alternaria* that three common pathogens that infect *Brassicaceae* (*A. brassicae*, *A. brassicicola*, and *A. japonica*) had almost no differences in their host ranges; nevertheless, they were distinctive in their conidial morphology and phylogenetic relationship to each other.

Alternaria cumini (sect. *Eureca*) and *A. triangularis*, which both infect *Apiaceae*, were morphologically and phylogenetically distinguishable from the other species, including *A. dauci* (sect. *Porri*), and selectively pathogenic to each original host genus (Table 5.6). In sect. *Radicina*, the morphology and pathogenicity of *A. petroselini* were also easily distinguishable from those of related species, except *A. selini* (Nishikawa & Nakashima 2013). It was appropriate to synonymize *A. selini* with *A. petroselini* based on multi-locus phylogenic similarities. According to Park *et al.* (2008), two species pathogenic to *Daucus*, *A. radicina* and *A. carotiincultae*, were phylogenetically recognized as a distinct species based on their *Alt a 1*, *tef1*, and β-tubulin gene sequences, but not on their *gapdh* and *rpb2* sequences (Woudenberg *et al.* 2013). Further

studies based on the integrated species recognition will be needed to define the species boundary.

In sect. *Gypsophilae*, Japanese isolates and a representative isolate of *A*. *nobilis* were clustered into a single lineage together with ex-type isolates of *A*. *ellipsoidea* and *A*. *saponariae* (Fig. 5.2). Conidial morphology of these closely related species are not clearly distinguished from each other, and it may be appropriate to regard them as one species, but phenotyping with host range evaluations are needed to define the species boundaries of *Dianthus* pathogens.

In sect. *Panax*, Japanese isolates and three representative isolates were clustered into a single lineage together with *A*. *dendropanacis* with strong BS support; however, this clade was divided into three subclades with lower BS support (Fig. 5.2), which were assigned to *A*. *panax*, *A*. *araliae*, and *A*. *dendropanacis* by Deng *et al.* (2015). Based on its conidial morphology and variability, and the culture characteristics of Japanese isolates, these features were not identical to the definition presented by Deng *et al.* (2015); however, it must be verified by detailed further studies.

In the present study, five Japanese species were clearly recognized in sect. *Porri*. Morphological distinctions based on the color of their beaks was an especially effective diagnostic feature. Among these, Woudenberg *et al.* (2014) phylogenetically differentiated *A*. *porri* from *A*. *allii*, and Japanese isolates identified as *A*. *porri* were clustered across the two species with lower BS support (Fig. 5.2). However, it will not be evident whether these two distinct species are recognizable as pathogens of *Allium* until additional phenotypings, such as host range, are applied. This section is also the largest, containing 63 species (Woudenberg *et al.* 2014), wherein phenotypings with both detailed morphological examinations and experimental host range are not sufficient. The ubiquitousness of *A*. *porri*, which was also shown in the present study, also demonstrated that integrated species recognition is strongly recommended to define the species boundaries of this section.

Thus far, we have discussed the utility of phenotyping based on experimental host ranges to distinguish closely related species. However, since the former *Ulocladium* species (*A*. *atra*, *A*.

botrytis, and *A*. *chartarum*) and one of the most frequent saprophytes, *A*. *alternata*, were commonly established as saprophytic isolates, it is difficult to examine their pathogenicity. Other approaches, such as secondary metabolites assays, are required to determine species boundaries among non-pathogenic species.

As a result of comprehensive inoculation tests, distinctive host selectivities were found along with the systematic ranks of each host plant, not only with genus but also subfamily, tribe, species, and variety, for the most highly plant-pathogenic species of *Alternaria*. Therefore, phenotypings with experimental host ranges reflect each species boundary based on morphology and molecular phylogeny. It was concluded that integrated species recognition based on morphology, phylogeny, and pathogenicity makes the species boundaries of the genus *Alternaria* clearer, and will provide a practical, re-defined species concept of the genus *Alternaria*.

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Chapter 6

General discussion and conclusion

SPECIES DIVERSITY IN JAPAN

In the present study, 26 *Alternaria* species have been described. The results present the biodiversity of *Alternaria* species in Japan, where at least 14 *Alternaria* sections are distributed (Fig. 5.2). Although relatively few Japanese *Alternaria* species were examined in the present study, they account for a wide range of recognized phylogenetic lineages and include three novel species, including *A. cylindrica*, *A. paragomphrenae*, and *A. triangularis*. In addition, information on other species only found in Japan, such as *A. petasitis* M. Kubota, Kishi & Abiko and *A. steviae* Ishiba, T. Yokoy. & Tani, which are unexamined in this study, are presented (Table 1.1). The results also suggest that potentially new species that are indigenous to Japan remain undiscovered.

Although *A. cinerariae*, *A. gaisen*, *A. gomphrenae*, and *A. iridicola* have been recorded in Japan for approximately 100 years (Nagano 1920; Togashi 1926; Shimazaki 1930; Enjoji 1931), their distribution remains limited, which could be influenced by the geographical distribution of their host plants and horticultural preferences in Japan. Consequently, the abovementioned species localized in Japan also characterize the peculiarities of the present *Alternaria* mycoflora in Japan. In contrast, five species, including *A. alstroemeriae*, *A. celosiicola*, *A. crassa*, *A. cumini*, and *A. petroselini*, are newly introduced species. *Alternaria panax*, which is found in *Polyscias* in Ogasawara (Bonin) Islands (Ono 2004), 1000 km away from Tokyo, could be considered an invasive species. *Polyscias* spp. are also invasive plants and are usually planted as garden trees

in the Islands, while *A. panax* is surprisingly widely distributed in Asia and North America (Deng *et al*. 2013). Therefore, it is plausible that *A. panax* could have been unintentionally spread to such an isolated area together with its host. This way, *Alternaria* fungi are able to fairly extend their geographical distribution. In addition, increasing levels of trade in plants could accelerate the spread of most of the plant-parasitic species, and commoditize their geographical isolations.

INTEGRATED SPECIES RECOGNITION TOWARD FUNGAL TAXONOMY IN THE FUTURE

Molecular phylogeny has been established as an important technique for fungal taxonomy. This technique, based on statistical analysis, is objective and can be used for evaluating the acceptability of conventional morphology-based taxonomy. It follows a split species concept and can be used for identifying cryptic species that cannot be distinguished on the basis of morphology alone. However, no phylogenetic species have been characterized by phenotypes that help us to understand the species concept. For example, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. and *C. acutatum* J.H. Simmonds, which were previously known as important plant pathogenic species with wide host ranges, were split into 20 more phylogenetic species on the basis of multi-locus phylogenetic analyses (Damm *et al.* 2012; Dean *et al*. 2012; Weir *et al.* 2012). However, the boundaries among these subdivided species have not yet been linked with their host ranges (Tomioka *et al.* 2013). *Cercospora* species are also important plant pathogens that were previously classified on the basis of their morphology and host plant genera (Chupp 1954). Although *Cercospora* species were long believed to be strictly host-selective, some isolates within *C. apii* sensu lato were found to have quite wide experimental host ranges (Akashi *et al.* 2008). Groenewald *et al.* (2012) redefined the species concept of *C. apii* Fresen. and allied species based on multi-locus phylogenetic analyses, but they were not able to associate that concept with host selectivity.

For these reasons, phenotyping with the analysis of pathogenicity has become more essential, especially for plant pathogenic taxa, while fungal taxonomy is developing with molecular phylogeny. Morphology- and host genera-based taxonomy in the genus *Alternaria* is also problematic, and the latest relevant molecular phylogenic study by Woudenberg *et al.* (2013) shows the necessity of additional phenotyping of *Alternaria* species boundaries.

Most of the species described in this study were well-characterized using the integrated species recognition method. As a result of comprehensive inoculation tests, distinctive host selectivities were identified along with the systematic rank of each host plant, including not only genus but also family, subfamily, tribe, species, and variety for most of the plant pathogenic species. It was concluded that integrated species recognition based on morphology, phylogeny, and pathogenicity makes species boundaries clearer and could provide new perspectives on the taxonomy of a wide range of plant pathogenic fungi.

PATHOGENICITY AND EVOLUTION

It has been previously reported that host-selective toxin (HST) producing strains referred to as *A. alternata* pathotypes could have acquired pathogenicity through the horizontal transfer of conditionally dispensable (CD) chromosome encoding HST biosynthetic gene clusters (Tsuge *et al*. 2013). In the present study, three *A. alternata*-like strains producing HSTs with a common epoxy-decatrienoic acid moiety were recognized as a distinct species, *A. gaisen* pathogenic to *Rosaceae* (strawberry and Japanese pear), except *A. alternata* f. sp. *citri* pathotype tangerine based on conidial morphology and phylogenetic analyses (Table 3.1; Fig. 3.3). Although the results partially supported the horizontal transfer of a CD chromosome between *A. alternata* and *A. gaisen*, they suggested that horizontal gene transfer contributes less to species specialization in *Alternaria* evolution. As a matter of fact, species in sect. *Alternaria*, including *A. alstroemeriae*, *A. cylindrica*, *A. iridicola*, and *A. longipes* (Ellis & Everh.) E.W. Mason are closely related to *A. alternata*; however, they are clearly distinguished at species level based on

morphology, molecular phylogeny, and host ranges. With regard to the two highly distinct species on *Brassicaceae* according to Parada *et al*. (2007), the pathogenicity of *A*. *brassicae* and *A*. *brassicicola*, which produce varying HSTs (ABR toxin and AB toxin, respectively) but exhibit almost identical host ranges (Table 5.5), could be a case of convergent evolution. Consequently, it was concluded that *Alternaria* has acquired host-selective pathogenicity in the course of evolution not only as a result of random and temporal horizontal gene transfer but also due to specialization by different species.

Generally, *Alternaria* sections exhibit host family-selectivity, including species in sect. *Alternantherae* are pathogenic to *Amaranthaceae*, sect. *Crivellia* are pathogenic to *Papaveraceae*, sect. *Gypsophilae* are pathogenic to *Caryophyllaceae*, sect. *Panax* are pathogenic to *Araliaceae*, sect. *Radicina* are pathogenic to *Apiaceae*, and sect. *Sonchi* are pathogenic to *Asteraceae* (Fig. 5.2). However, sect. *Alternaria* and *Porri* exhibit pathogenicity to multi-host families, especially sect. *Porri* is pathogenic to *Amaryllidaceae*, *Apiaceae*, *Asteraceae*, *Cucurbitaceae*, and *Solanaceae* and they could still exhibit subspecialization toward single- to multi-host genera, species, or varieties. Therefore, phenotyping and the determination of host ranges, in addition to other approaches, such as studies on toxin profiles and pathogenicity-associated genes would be required to understand the evolution of *Alternaria* and its pathogenic specialization. The author envisages undertaking further studies on pathogenic specialization in sect. *Porri*.

TAXONOMIC NOVELTIES

New species: *Alternaria cylindrica* Jun. Nishikawa & C. Nakash., *Alternaria paragomphrenae* Jun. Nishikawa & C. Nakash., *Alternaria triangularis* Jun. Nishikawa & C. Nakash.

New name: *Alternaria celosiicola* Jun. Nishikawa & C. Nakash.

Verified name: *Alternaria gaisen* Nagano ex Bokura

Typifications:

Lectotype: *Alternaria gomphrenae* Togashi

Epitype: *Alternaria cinerariae* Hori & Enjoji, *Alternaria gaisen* Bokura, *Alternaria gomphrenae* Togashi, *Alternaria iridicola* (Ellis & Everh.) J.A. Elliott, *Alternaria japonica* Yoshii

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Summary

Alternaria is well known as one of the most ubiquitous fungus genera, inhabiting every environmental substrate, but actually consists of mostly plant parasitic species. Especially for vegetables and ornamental flowers, these fungi are often regarded as seed-borne pathogens that cause large economic losses; therefore, they are very important for agriculture and seed production. In previous taxonomies of *Alternaria*, morphological features especially conidial shapes and sporulation patterns were the focus of species keys. However, this made species recognition complicated and confusing because of their morphological variation and fundamental pleomorphism. Moreover, the use of host plants as an additional taxonomic key made matters worse and resulted in the genus being split into more than 400 species. The latest molecular phylogenies have drastically reconstructed *Alternaria* and allied genera, and reconsidered several species, but the relationships between taxonomy and plant parasitism remain unclear. In addition, comprehensive studies on the distribution and biodiversity of Japanese species of the genus *Alternaria* have been inconclusive. Therefore, 85 alternarioid isolates obtained through field surveys by the author and from several collaborators or public culture collections were used for morphological and cultural comparisons, and molecular phylogenetic analyses using multi-locus DNA sequences (ITS, *actin*, *Alt-a 1*, *endoPG*, *gapdh*, *rpb2*, and *tef1*). This thesis proposed integrated species criteria for the taxonomy of the genus *Alternaria* based on morphology, molecular phylogeny, and pathogenicity. To make species boundaries clearer, systematic pathological phenotyping, namely determining the experimental host ranges of each species, was conducted.

To reveal morphological variability and pleomorphism within an *Alternaria* species, detailed morphological comparisons of three isolates of *A. cinerariae* with different source host plants were performed. The results suggest that *A. cinerariae* has considerable morphological variation with respect to sporulation patterns, conidial swelling, and chlamydospore formation, and provide an example of the high variability within *Alternaria* species. Phylogenetic analysis using *gapdh*, *rpb2*, and *tef1* sequences clustered the isolates together in a single clade and

supported the above conclusion. In addition, inoculation tests on 17 species produced etiologically interesting results indicating that the experimental host range of *A*. *cinerariae* is mostly selective to one tribe, *Senecioneae*.

The strawberry black leaf spot pathogen is one of the most host-selective pathogens in the genus *Alternaria*. This pathogen, which is known to be host variety-selective, has been identified as *A*. *alternata*, which is also known to be a highly confusable morphological taxon. Based on morphological observations and phylogenetic analysis of a combined dataset of ITS, *gapdh*, *rpb2*, *tef1*, *Alt a 1*, and *endoPG* sequences, the examined isolates were identified as *A*. *gaisen* rather than *A*. *alternata* together with isolates of Japanese pear pathotypes of *A*. *alternata*. Furthermore, *A*. *gaisen* was taxonomically re-examined and re-described as *A*. *gaisen* Nagano ex Bokura, which includes two formae speciales, *A*. *gaisen* f. sp. *fragariae* producing AF-toxin and f. sp. *pyri* producing AK-toxin.

To phenotype four species new to Japan, *A*. *alstroemeriae*, *A*. *celosiicola*, *A*. *crassa*, and *A*. *petroselini*, an integrated species recognition method based on morphology, phylogenetic analysis using ITS sequences, and experimental host range was tested. Inoculation tests on different species revealed that these species had selective host ranges limited to one genus (*A*. *alstroemeriae*), two related tribes (*A*. *crassa*) or two subfamilies (*A*. *celosiicola*), or were widely pathogenic within one family (*A*. *petroselini*. The results also suggested that integrated species criteria are helpful to find potential susceptible hosts to individual *Alternaria* species and to recognize species boundaries between related species, especially for plant pathologists.

Furthermore, the integrated species recognition method was extended to some known species distributed in Japan to clarify their species boundaries, focusing on phylogenetically, morphologically, or pathologically closely related species. The experimental host range results suggested that species in sect. *Alternantherae*, which includes *A*. *celosiicola*, *A*. *gomphrenae*, and a novel species named *A*. *paragomphrenae*, could be clearly differentiated by pathogenicity to *Amaranthaceae* plants, reflecting their morphological and phylogenetic differences. Conversely, the experimental host ranges of three morphologically and phylogenetically distinct species pathogenic on *Brassicaceae*, *A*. *brassicae*, *A*. *brassicicola*, and

A. *japonica*, interestingly showed almost no differences in host selectivity. For the other obtained species including *A*. *iridicola* pathogenic to *Iris* spp. (*Iridaceae*) and *A*. *cumini* pathogenic to *Cuminum* (*Apiaceae*), species boundaries were determined based on integrated species recognition to discuss the validity of the concept.

During this study, 85 isolates were obtained and described as 26 species, which included three novel species and five new records to Japan, with several taxonomic treatments. Comprehensive inoculation tests revealed distinctive host selectivities along with the systematic ranks of the host plants, including not only genus but also tribe, subfamily, and variety, for most plant-pathogenic species of *Alternaria*. Therefore, phenotyping using experimental host ranges can reflect species boundaries. It was concluded that integrated species recognition based on morphology, phylogeny, and pathogenicity can make species boundaries clearer and will provide a practical way to re-define the species concept in the genus *Alternaria*.

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