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The *Colletotrichum destructivum* species complex – hemibiotrophic pathogens of forage and field crops

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Abstract: *Colletotrichum destructivum* is an important plant pathogen, mainly of forage and grain legumes including clover, alfalfa, cowpea and lentil, but has also been reported as an anthracnose pathogen of many other plants worldwide. Several *Colletotrichum* isolates, previously reported as closely related to *C. destructivum*, are known to establish hemibiotrophic infections in different hosts. The inconsistent application of names to those isolates based on outdated species concepts has caused much taxonomic confusion, particularly in the plant pathology literature. A multilocus DNA sequence analysis (ITS, GAPDH, CHS-1, HIS3, ACT, TUB2) of 83 isolates of *C. destructivum* and related species revealed 16 clades that are recognised as separate species in the *C. destructivum* complex, which includes *C. destructivum*, *C. fuscum*, *C. higginsianum*, *C. lini* and *C. tabacum*. Each of these species is lecto-, epi- or neotypified in this study. Additionally, eight species, namely *C. americae-borealis*, *C. antirrhinicola*, *C. bryoniicola*, *C. lentis*, *C. ocimi*, *C. pisicola*, *C. utrechtense* and *C. vignae* are newly described.

Key words: Anthracnose, *Ascomycota*, *Glomerella*, Phylogenetics, Systematics.

Taxonomic novelties: New species: *Colletotrichum americae-borealis* Damm, *C. antirrhinicola* Damm, *C. bryoniicola* Damm, *C. lentis* Damm, *C. ocimi* Damm, *C. pisicola* Damm, *C. utrechtense* Damm, *C. vignae* Damm; **Typifications: Epitypifications (basionyms):** *C. destructivum* O'Gara, *C. fuscum* Laubert, *C. higginsianum* Sacc., *Gloeosporium lini* Westerd; **Lectotypifications (basionyms):** *C. fuscum* Laubert, *Gm. lini* Westerd., *C. pisi* Pat; **Neotypification (basionym):** *C. tabacum* Böning.

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INTRODUCTION

Colletotrichum destructivum was originally described as the causal organism of a disease of clover (*Trifolium pratense* and *T. hybridum*) in the western USA (O'Gara 1915). To date this species has been reported from more than 30 hosts belonging to at least 11 plant families, the majority of them being *Fabaceae* (especially *Trifolium*, *Medicago*, *Glycine*), but also including several reports from *Poaceae* (especially *Phalaris*, *Triticum*) and a few reports from *Asteraceae* (*Chrysanthemum*), *Convolvulaceae* (*Cuscuta*), *Magnoliaceae* (*Michelia*), *Menispermaceae* (*Cocculus*), *Polygonaceae* (*Rumex*), *Solanaceae* (*Nicotiana*), *Lamiaceae* (*Perilla*), *Scophulariaceae* (*Antirrhinum*, *Sutera*) and *Orchidaceae* (*Bletilla*). These reports originate from 18 countries, mainly in North America, Asia and Africa; with comparatively few reports from Europe, South America and Oceania (Kawaradani *et al.* 2008, Tomioka *et al.* 2011, 2012, Farr & Rossman 2014).

According to Sutton (1992), conidia of *C. destructivum* measure 10–22 × 4–6 µm, are straight to slightly curved, abruptly tapered to an obtuse apex and a truncate base, while according to Baxter *et al.* (1983) they are much narrower, measuring 16–18 × 3 µm, mostly straight and have tapered ends.

Since many other *Colletotrichum* species are also known from the host plants listed above, there is confusion about the names applied to different collections. For example, Cannon *et al.* (2012) found that half of the ITS sequences of *C. trifolii* submitted to GenBank prior to their study, were based on misidentified strains that actually belonged to the *C. destructivum* complex. Many isolates assigned to the *C. destructivum* species complex in a preliminary phylogeny based on ITS and included in this study for further analysis, had previously been identified as *C. coccodes*, *C. lindemuthianum*, *C. trifolii*, *C. truncatum*, *C. gloeosporioides* or *Glomerella cingulata* var. *cingulata*. Further confusion was caused by connecting *C. destructivum* to the sexual morph *Ga. glycines* (Tiffany & Gilman 1954, Manandhar *et al.* 1986), which was originally described by Lehman & Wolf (1926) from soybean stems as the sexual morph of *C. glycines*. In contrast, von Arx & Müller (1954) treated *Ga. glycines* as a form of *Ga. cingulata* with large ascospores.

A number of species were observed to have a similar morphology to *C. destructivum* and were considered to be closely related to that species. In the study of Moriwaki *et al.* (2002), Japanese *Colletotrichum* isolates clustered into 20 groups based on ITS1 sequences, which correlated with their morphology; isolates of *C. destructivum*, *C. fuscum*, *C. higginsianum* and *C. linicola* belonged to the same ribosomal group and were considered as possibly conspecific. Based on D2 and ITS2 rDNA sequences, Latunde-Dada & Lucas (2007) found a close relationship among *C. destructivum* isolates from *Vigna unguiculata* and *Medicago sativa*, *C. linicola* isolates from *Linum* and *C. truncatum* isolates from *Pisum sativum*, *Vicia faba*

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and *Lens culinaris*, which clustered with *C. higginsianum* isolates in their phylogeny. Based on multilocus phylogenies, *C. destructivum* was recently delineated as a species complex with *C. fuscum*, *C. higginsianum*, *C. tabacum*, *C. linicola* and *Ga. truncata* (Cannon *et al.* 2012, O'Connell *et al.* 2012). However, only a few isolates were included in those studies.

The infection strategy of isolates from several hosts of *C. destructivum* and related species has been reported as hemibiotrophic (Bailey *et al.* 1992, O'Connell *et al.* 1993, Shen *et al.* 2001) and several genes involved in plant infection have been studied (Huser *et al.* 2009, Kleemann *et al.* 2012, Liu *et al.* 2013b). To better understand the molecular basis of the infection process, O'Connell *et al.* (2012) compared genome and transcriptome sequence data of *C. higginsianum* with those of *C. graminicola*, a hemibiotrophic species from a different *Colletotrichum* species complex. This study revealed that both species possessed unusually large sets of pathogenicity-related genes, combining features of both biotrophic and necrotrophic pathogens. In particular, genes encoding plant cell wall-degrading enzymes, proteases and secondary metabolism enzymes are all expanded, similar to necrotrophs, but these fungi also encode large numbers of effector proteins for host manipulation, more similar to biotrophs. Transcriptome sequencing showed that expression of these genes is highly stage-specific, with most effector and secondary metabolism genes expressed early during appressorial penetration and biotrophy, and most plant cell wall-degrading enzymes, proteases and nutrient uptake transporters induced later at the switch to necrotrophy.

Prior to this study, the phylogenetic relationships of species in the *C. destructivum* complex have been studied inadequately using modern molecular methods. Many species names in this complex have been applied inconsistently or incorrectly, as there have been no recent studies of type specimens and few ex-type cultures are available for sequence analyses. Preliminary results based on multilocus DNA sequences of a small dataset indicated that isolates from different hosts belonged to several closely related species. The aim of our study was to recollect, delineate, typify and characterise the species within the *C. destructivum* complex, based on multilocus DNA sequence and morphological data.

MATERIALS AND METHODS

Isolates

A total of 83 isolates from the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, the Netherlands, and other culture collections was studied, most of which had been previously identified as *C. destructivum*. Type specimens (holo-, lecto-, epi- and neotypes) of the species studied are located in the fungaria of the CBS, the US National Fungus Collections (BPI), Beltsville, Maryland, USA, the Royal Botanic Gardens, Kew, UK, (IMI and K(M)), and the Botanic Garden and Botanical Museum Berlin-Dahlem, Freie Universität Berlin (B), Germany. All descriptions are based on ex-holotype, ex-epitype or ex-neotype cultures as applicable. Features of other isolates or specimens are included if they deviate from the ex-type cultures. Subcultures of the holo-, epi- and neotypes as well as all other isolates used for morphological and sequence analyses are maintained in the culture collections listed in Table 1.

Morphological analysis

To enhance sporulation, autoclaved filter paper and double-autoclaved stems of *Anthriscus sylvestris* were placed onto the surface of synthetic nutrient-poor agar medium (SNA; Nirenberg 1976). SNA and OA (oatmeal agar; Crous *et al.* 2009) cultures were incubated at 20 °C under near-UV light with a 12 h photoperiod for 10 d. Measurements and photomicrographs of characteristic structures were made according to Damm *et al.* (2007). Appressoria were observed on the reverse side of SNA plates. Microscopic preparations were made in clear lactic acid, with 30 measurements per structure and observed with a Nikon SMZ1000 dissecting microscope (DM) or with a Nikon Eclipse 80i microscope using differential interference contrast (DIC) illumination.

Colony characters and pigment production on SNA and OA cultures incubated at 20 °C under near-UV light with a 12 h photoperiod were noted after 10 d. Colony colours were rated according to Rayner (1970). Growth rates were measured after 7 and 10 d.

Phylogenetic analysis

Genomic DNA of the isolates was extracted using the method of Damm *et al.* (2008). The ITS, GAPDH, and partial sequences of the chitin synthase 1 (CHS-1), histone H3 (HIS3), actin (ACT) and beta-tubulin (TUB2) genes were amplified and sequenced using the primer pairs ITS-1F (Gardes and Bruns 1993) + ITS-4 (White *et al.* 1990), GDF1 + GDR1 (Guerber *et al.* 2003), CHS-354R + CHS-79F (Carbone & Kohn 1999), CYLH3F + CYLH3R (Crous *et al.* 2004b), ACT-512F + ACT-783R (Carbone & Kohn 1999) and T1 (O'Donnell & Cigelnik 1997) + Bt-2b (Glass & Donaldson 1995) or T1 + BT4R (Woudenberg *et al.* 2009), respectively. The PCRs were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, California) in a total volume of 12.5 µL. The GAPDH, CHS-1, HIS3, ACT and TUB2 PCR mixture contained 1 µL 20× diluted genomic DNA, 0.2 µM of each primer, 1× PCR buffer (Bioline, Luckenwalde, Germany), 2 mM $MgCl_2$, 20 µM of each dNTP, 0.7 µL DMSO and 0.25 U Taq DNA polymerase (Bioline). Conditions for PCR of these genes constituted an initial denaturation step of 5 min at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 52 °C and 30 s at 72 °C, and a final denaturation step of 7 min at 72 °C, while the ITS PCR was performed as described by Woudenberg *et al.* (2009). The DNA sequences generated with forward and reverse primers were used to obtain consensus sequences using Bionumerics v. 4.60 (Applied Maths, St-Marthens-Lathem, Belgium), and the alignment assembled and manually adjusted using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002).

To determine whether the six sequence datasets were congruent and combinable, tree topologies of 70 % reciprocal Neighbour-Joining bootstrap with Maximum Likelihood distances (10 000 replicates) with substitution models determined separately for each partition using MrModeltest v. 2.3 (Nylander 2004) were compared visually (Mason-Gamer and Kellogg 1996). A maximum parsimony analysis was performed on the multilocus alignment (ITS, GAPDH, CHS-1, HIS3, ACT, TUB2) as well as for each gene separately with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) using the heuristic search option with 100 random sequence additions and tree bisection and reconstruction (TBR) as the branch-swapping

Table 1. Strains of *Colletotrichum* spp. studied, with collection details and GenBank accession numbers.

Species	Accession No.[1]	Host	Country	GenBank No.[2]					
				ITS	GAPDH	CHS-1	HIS3	ACT	TUB2
C. americae-borealis	CBS 136232*	*Medicago sativa*	USA	**KM105224**	**KM105579**	**KM105294**	**KM105364**	**KM105434**	**KM105504**
	CBS 136855	*Medicago sativa*	USA	**KM105225**	**KM105580**	**KM105295**	**KM105365**	**KM105435**	**KM105505**
	ATCC 11869, LARS 373, CPC 18946	*Medicago sativa*	USA	**KM105223**	**KM105578**	**KM105293**	**KM105363**	**KM105433**	**KM105503**
C. antirrhinicola	CBS 102189*	*Antirrhinum majus*	New Zealand	**KM105180**	**KM105531**	**KM105250**	**KM105320**	**KM105390**	**KM105460**
C. bryoniicola	CBS 109849*	*Bryonia dioica*	Netherlands	**KM105181**	**KM105532**	**KM105251**	**KM105321**	**KM105391**	**KM105461**
C. destructivum	CBS 114801, AR 4031	*Crupina vulgaris*	Greece	**KM105219**	**KM105574**	**KM105289**	**KM105359**	**KM105429**	**KM105499**
	CBS 119187, AR 4031	*Crupina vulgaris*	Greece	**KM105220**	**KM105575**	**KM105290**	**KM105360**	**KM105430**	**KM105500**
	CBS 128509, LARS 320	*Medicago sativa*	Canada	**KM105214**	**KM105569**	**KM105284**	**KM105354**	**KM105424**	**KM105494**
	CBS 157.83	*Medicago sativa*	Serbia	**KM105215**	**KM105570**	**KM105285**	**KM105355**	**KM105425**	**KM105495**
	CBS 511.97, LARS 202	*Medicago sativa*	Morocco	**KM105216**	**KM105571**	**KM105286**	**KM105356**	**KM105426**	**KM105496**
	CBS 520.97, LARS 709	*Medicago sativa*	Saudi Arabia	**KM105217**	**KM105572**	**KM105287**	**KM105357**	**KM105427**	**KM105497**
	CBS 167.58	*Medicago sativa*	Italy	**KM105213**	**KM105568**	**KM105283**	**KM105353**	**KM105423**	**KM105493**
	CBS 130238, 5/5/11-1-1	*Phragmites*	USA	**KM105218**	**KM105573**	**KM105288**	**KM105358**	**KM105428**	**KM105498**
	IMI 387103, CPC 18082	*Rumex* sp.	Korea	**KM105221**	**KM105576**	**KM105291**	**KM105361**	**KM105431**	**KM105501**
	CBS 136228*	*Trifolium hybridum*	USA	**KM105207**	**KM105561**	**KM105277**	**KM105347**	**KM105417**	**KM105487**
	CBS 136852	*Trifolium hybridum*	USA	**KM105208**	**KM105562**	**KM105278**	**KM105348**	**KM105418**	**KM105488**
	CBS 136853	*Trifolium hybridum*	USA	**KM105209**	**KM105563**	**KM105279**	**KM105349**	**KM105419**	**KM105489**
	CBS 136229	*Trifolium hybridum*	USA	**KM105211**	**KM105565**	**KM105281**	**KM105351**	**KM105421**	**KM105491**
	CBS 136230	*Trifolium repens*	USA	**KM105210**	**KM105564**	**KM105280**	**KM105350**	**KM105420**	**KM105490**
	CBS 136231	*Trifolium repens*	USA	**KM105212**	**KM105566**	**KM105282**	**KM105352**	**KM105422**	**KM105492**
	CBS 149.34	*Trifolium* sp.	Netherlands	JQ005764	**KM105567**	JQ005785	JQ005806	JQ005827	JQ005848
C. fuscum	CBS 133704	*Digitalis dubia*	Netherlands	**KM105176**	**KM105526**	**KM105246**	**KM105316**	**KM105386**	**KM105456**
	CBS 130.57	*Digitalis lanata*	unknown	JQ005762	**KM105530**	JQ005783	JQ005804	JQ005825	JQ005846
	CBS 133701*	*Digitalis lutea*	Germany	**KM105174**	**KM105524**	**KM105244**	**KM105314**	**KM105384**	**KM105454**
	CBS 133702	*Digitalis lutea*	Netherlands	**KM105178**	**KM105528**	**KM105248**	**KM105318**	**KM105388**	**KM105458**
	CBS 133703	*Digitalis obscura*	Netherlands	**KM105175**	**KM105525**	**KM105245**	**KM105315**	**KM105385**	**KM105455**
	CBS 825.68	*Digitalis purpurea*	Netherlands	**KM105177**	**KM105527**	**KM105247**	**KM105317**	**KM105387**	**KM105457**
	CBS 200.54	unknown	Germany	**KM105179**	**KM105529**	**KM105249**	**KM105319**	**KM105389**	**KM105459**
C. higginsianum	Abc 6-2, CPC 19368	*Brassica chinensis*	Japan	**KM105187**	**KM105539**	**KM105257**	**KM105327**	**KM105397**	**KM105467**
	IMI 349061, CPC 19379*	*Brassica chinensis*	Trinidad and Tobago	**KM105184**	**KM105535**	**KM105254**	**KM105324**	**KM105394**	**KM105464**
	IMI 349063, CPC 19380	*Brassica chinensis*	Trinidad and Tobago	JQ005760	**KM105536**	JQ005781	JQ005802	JQ005823	JQ005844
	Abo 1-1, CPC 19364	*Brassica oleracea* Gemmifera group	Japan	**KM105185**	**KM105537**	**KM105255**	**KM105325**	**KM105395**	**KM105465**
	Abp 1-2, CPC 19365	*Brassica pekinensis*	Japan	**KM105186**	**KM105538**	**KM105256**	**KM105326**	**KM105396**	**KM105466**
	Abr 2-2, CPC 19369	*Brassica rapa*	Japan	**KM105188**	**KM105540**	**KM105258**	**KM105328**	**KM105398**	**KM105468**
	Abr 3-1, CPC 19370	*Brassica rapa*	Japan	**KM105189**	**KM105541**	**KM105259**	**KM105329**	**KM105399**	**KM105469**
	MAFF 305635, Abr 1-5, CPC 19366	*Brassica rapa* Perviridis Group	Japan	JQ005761	**KM105542**	JQ005782	JQ005803	JQ005824	JQ005845
	CBS 128508, LARS 889, Kyoto 337-5	*Brassica rapa* var. *komatsuna*	Japan	**KM105190**	**KM105543**	**KM105260**	**KM105330**	**KM105400**	**KM105470**
	NBRC 6182, CPC 18944	*Brassica* sp.	Italy	**KM105191**	**KM105544**	**KM105261**	**KM105331**	**KM105401**	**KM105471**
	AR 3-5, CPC 19363	*Raphanus sativus*	Japan	**KM105192**	**KM105545**	**KM105262**	**KM105332**	**KM105402**	**KM105472**
	AR 3-1, CPC 19394	*Raphanus sativus*	Japan	**KM105193**	**KM105546**	**KM105263**	**KM105333**	**KM105403**	**KM105473**
	AR 7-3, CPC 19395	*Raphanus sativus* var. *sativus*	Japan	**KM105194**	**KM105547**	**KM105264**	**KM105334**	**KM105404**	**KM105474**
	AR 8-1, CPC 19396	*Raphanus sativus*	Japan	**KM105195**	**KM105548**	**KM105265**	**KM105335**	**KM105405**	**KM105475**

(*continued on next page*)

Table 1. (Continued).

Species	Accession No.[1]	Host	Country	GenBank No.[2]					
				ITS	GAPDH	CHS-1	HIS3	ACT	TUB2
C. lentis	CBS 127604, DAOM 235316, CT21*	*Lens culinaris*	Canada	JQ005766	**KM105597**	JQ005787	JQ005808	JQ005829	JQ005850
	CBS 127605, DAOM 235317, CT26	*Lens culinaris*	Canada	**KM105241**	**KM105598**	**KM105311**	**KM105381**	**KM105451**	**KM105521**
C. lini	CBS 172.51*	*Linum usitatissimum*	Netherlands	JQ005765	**KM105581**	JQ005786	JQ005807	JQ005828	JQ005849
	CBS 505.97, LARS 77	*Linum usitatissimum*	Ireland	**KM105226**	**KM105582**	**KM105296**	**KM105366**	**KM105436**	**KM105506**
	IMI 103842, CPC 18947	*Linum usitatissimum*	UK	**KM105227**	**KM105583**	**KM105297**	**KM105367**	**KM105437**	**KM105507**
	IMI 103844, CPC 16816	*Linum usitatissimum*	UK	**KM105228**	**KM105584**	**KM105298**	**KM105368**	**KM105438**	**KM105508**
	CBS 112.21, LCP 46.621	*Linum usitatissimum*	UK	**KM105229**	**KM105585**	**KM105299**	**KM105369**	**KM105439**	**KM105509**
	CBS 100569, PD 97/ 14304	*Nigella* sp.	France	**KM105230**	**KM105586**	**KM105300**	**KM105370**	**KM105440**	**KM105510**
	IMI 391904, IS320, CPC 19382	*Raphanus raphanistrum*	Tunisia	**KM105232**	**KM105588**	**KM105302**	**KM105372**	**KM105442**	**KM105512**
	CBS 117156	*Teucrium scorodonia*	Netherlands	**KM105231**	**KM105587**	**KM105301**	**KM105371**	**KM105441**	**KM105511**
	CBS 136856	*Medicago sativa*	USA	**KM105233**	**KM105589**	**KM105303**	**KM105373**	**KM105443**	**KM105513**
	CBS 136857	*Taraxacum* sp.	USA	**KM105239**	**KM105595**	**KM105309**	**KM105379**	**KM105449**	**KM105519**
	CBS 136233	*Taraxacum* sp.	USA	**KM105240**	**KM105596**	**KM105310**	**KM105380**	**KM105450**	**KM105520**
	CBS 136850	*Trifolium hybridum*	USA	**KM105237**	**KM105593**	**KM105307**	**KM105377**	**KM105447**	**KM105517**
	CBS 136851	*Trifolium hybridum*	USA	**KM105238**	**KM105594**	**KM105308**	**KM105378**	**KM105448**	**KM105518**
	CBS 130828	*Trifolium repens*	Germany	**KM105234**	**KM105590**	**KM105304**	**KM105374**	**KM105444**	**KM105514**
	CBS 130829	*Trifolium repen*	Germany	**KM105235**	**KM105591**	**KM105305**	**KM105375**	**KM105445**	**KM105515**
	IMI 69991, CPC 20242	*Trifolium* sp.	New Zealand	**KM105236**	**KM105592**	**KM105306**	**KM105376**	**KM105446**	**KM105516**
C. ocimi	CBS 298.94*	*Ocimum basilicum*	Italy	**KM105222**	**KM105577**	**KM105292**	**KM105362**	**KM105432**	**KM105502**
C. panacicola	C08087	*Panax ginseng*	Korea	GU935869	GU935849			GU944758	
	C08061	*Panax ginseng*	Korea	GU935868	GU935848			GU935791	
	C08048	*Panax ginseng*	Korea	GU935867	GU935847			GU944757	
C. pisicola	CBS 724.97, LARS 60*	*Pisum sativum*	USA	**KM105172**	**KM105522**	**KM105242**	**KM105312**	**KM105382**	**KM105452**
C. tabacum	CBS 124249, MUCL 44942	*Centella asiatica*	Madagascar	**KM105206**	**KM105560**	**KM105276**	**KM105346**	**KM105416**	**KM105486**
	N150, CPC 18945*	*Nicotiana tabacum*	Canada	**KM105204**	**KM105557**	**KM105274**	**KM105344**	**KM105414**	**KM105484**
	IMI 50187, CPC 16820	*Nicotiana tabacum*	India	**KM105205**	**KM105558**	**KM105275**	**KM105345**	**KM105415**	**KM105485**
	CBS 161.53	*Nicotiana tabacum*	Zambia	JQ005763	**KM105559**	JQ005784	JQ005805	JQ005826	JQ005847
C. tanaceti	CBS 132693, BRIP 57314, UM01*	*Tanacetum cinerariifolium*	Australia	JX218228	JX218243	JX259268		JX218238	JX218233
	CBS 132818, BRIP 57315, TAS060-0003	*Tanacetum cinerariifolium*	Australia	JX218229	JX218244	JX259269		JX218239	JX218234
	BRIP 57316, TAS060-0004	*Tanacetum cinerariifolium*	Australia	JX218230	JX218245	JX259270		JX218240	JX218235
C. utrechtense	CBS 130243*	*Trifolium pratense*	Netherlands	**KM105201**	**KM105554**	**KM105271**	**KM105341**	**KM105411**	**KM105481**
	CBS 135827	*Trifolium pratense*	Netherlands	**KM105202**	**KM105555**	**KM105272**	**KM105342**	**KM105412**	**KM105482**
	CBS 135828	*Trifolium pratense*	Netherlands	**KM105203**	**KM105556**	**KM105273**	**KM105343**	**KM105413**	**KM105483**
C. vignae	CBS 501.97, LARS 56*	*Vigna unguiculata*	Nigeria	**KM105183**	**KM105534**	**KM105253**	**KM105323**	**KM105393**	**KM105463**
	IMI 334960, CPC 19383	*Vigna unguiculata*	Nigeria	**KM105182**	**KM105533**	**KM105252**	**KM105322**	**KM105392**	**KM105462**
Colletotrichum sp.	CBS 125336	*Heracleum* sp.	Netherlands	**KM105198**	**KM105551**	**KM105268**	**KM105338**	**KM105408**	**KM105478**
	CBS 126510	*Heracleum* sp.	Netherlands	**KM105199**	**KM105552**	**KM105269**	**KM105339**	**KM105409**	**KM105479**
	CPC 18076	*Heracleum* sp.	Netherlands	**KM105200**	**KM105553**	**KM105270**	**KM105340**	**KM105410**	**KM105480**

Table 1. (Continued).

Species	Accession No.[1]	Host	Country	GenBank No.[2]					
				ITS	GAPDH	CHS-1	HIS3	ACT	TUB2
Colletotrichum sp.	CH90-M1, CPC 19361	*Matthiola incana*	Japan	**KM105196**	**KM105549**	**KM105266**	**KM105336**	**KM105406**	**KM105476**
	CH93-M1, CPC 19362	*Matthiola incana*	Japan	**KM105197**	**KM105550**	**KM105267**	**KM105337**	**KM105407**	**KM105477**
	CBS 107.40	*Pisum sativum*	Russia	**KM105173**	**KM105523**	**KM105243**	**KM105313**	**KM105383**	**KM105453**

*ex-holotype, ex-epitype or ex-neotype culture.

[1] ATCC: American Type Culture Collection, Virginia, USA; BRIP: Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; LARS: Culture collection of Long Ashton Research Station, Bristol, UK (no longer existing); MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MUCL: Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NBRC: Culture Collection of the Biological Resource Center, National Institute of Technology and Evaluation, Kisarazu, Japan; PD: Plantenziektenkundige Dienst, Wageningen, Netherlands.

[2] ITS: internal transcribed spacers and intervening 5.8S nrDNA; GAPDH: partial glyceraldehyde-3-phosphate dehydrogenase gene; CHS-1: partial chitin synthase-1 gene; HIS: partial histone H3 gene; ACT: partial actin gene; TUB2: partial beta-tubulin gene. Sequences generated in this study are emphasised in bold face.

algorithm. Alignment gaps were treated as new states and all characters were unordered and of equal weight. The robustness of the trees obtained was evaluated by 1 000 bootstrap replications using the same settings as for the parsimony analysis itself (Hillis & Bull 1993). Tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for the resulting trees. A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003) for the combined sequence datasets. Models of nucleotide substitution for each gene determined by the AIC criterion as implemented in MrModeltest v. 2.3 were included for each gene partition. The analyses of two parallel Markov Chain Monte Carlo (MCMC) runs, each consisting of four chains, were run from random trees for 100 M generations and sampled every 1 000 generations until the runs converged with a split frequency of 0.01. The first 25 % of trees were discarded as the burn-in phase of the analysis and posterior probabilities determined from the remaining trees. For additional comparison, a Neighbour-Joining analysis was performed on the multilocus alignment using PAUP with 10 000 bootstrap replications. Sequences derived in this study have been lodged at GenBank, the alignment and tree in TreeBASE (www.treebase.org/treebase-web/home.html) (S16069), and taxonomic novelties in MycoBank (Crous *et al.* 2004a).

RESULTS

Phylogeny

The six individual datasets did not show any conflicts in tree topology for the 70 % reciprocal bootstrap trees, which allowed us to combine them. In the multilocus analyses (gene boundaries of ITS: 1–560, GAPDH: 571–795, CHS-1: 806–1085, HIS3: 1096–1485, ACT: 1496–1758, TUB2: 1769–2281) of 83 isolates of *C. destructivum* and related *Colletotrichum* species and the outgroup (*C. pisicola* CBS 724.97 and *Colletotrichum* sp. CBS 107.40), 2 281 characters including the alignment gaps were processed, of which 349 characters were parsimony-informative, 48 parsimony-uninformative and 1 884 constant. After a heuristic search using PAUP, 14 equally most parsimonious trees were retained (tree length = 540 steps, CI = 0.828, RI = 0.962, RC = 0.796, HI = 0.172) of which the first tree is shown in Fig. 1. The overall topology of all of the equally most parsimonious trees was similar; they differed only in the position of isolates within the *C. destructivum s. str.* clade. The Bayesian analysis was conducted using the following substitution models: dirichlet (1,1,1,1) state frequency distributions were used for all loci except for CHS-1 which had a fixed (equal) state frequency distribution; for ITS the model was HKY with a proportion of invariable sites allowed, for both GAPDH and CHS-1 the model was HKY with an equal variation rate across sites, for HIS3 the model was GTR with a gamma-shaped rate variation across sites, for ACT the model was HKY with a gamma-shaped rate variation across sites, and for TUB2 the model was GTR with a proportion of invariable sites allowed. The Bayesian analysis lasted 1 081 000 generations, after which the split frequency reached less than 0.01; 1 622 trees of the 2 162 trees were used to calculate the consensus tree and posterior probabilities (PP's; see values plotted onto Fig. 1).

The analysis resulted in the delineation of seven main clades and 16 subclades within the *C. destructivum* species complex, which we accept as representing different *Colletotrichum* species. The first main clade (bootstrap support value = 91 %/ Bayesian posterior probability value = 1.00) consists of several closely related species including *C. fuscum* (74/1.00), *C. higginsianum* (74/1.00), *C. vignae* (99/1.00), two single isolates clades belonging to *C. anthirrhinicola* and *C. bryoniicola* and five unnamed strains. *Colletotrichum utrechtense* (78) and *C. panacicola* (95/1.00) belonged to the second main clade, while the third clade only contained one subclade, representing *C. tabacum* (100/1.00). Clade four consists of a large number of *C. destructivum s. str.* isolates (95/0.99) and a sister clade on a long branch representing *C. ocimi*. Clade five consists of two subclades representing *C. americae-borealis* (67/0.92) and *C. lini* (98/1.00). Clade six is represented by two well-supported subclades on long branches, *C. lentis* (100/1.00) and *C. tanaceti* (100/1.00). The seventh main clade consists of a long branch with two single strain clades representing *C. pisicola* and a second unidentified species from *Pisum* and is basal to the rest of the isolates and was consequently chosen as the outgroup of the phylogeny.

The consensus tree obtained from the Bayesian analysis and the NJ tree (not shown) confirmed the tree topology obtained from the parsimony analysis. Bayesian posterior probability values mostly agreed with bootstrap support values and are also plotted on the phylogram (Fig. 1). The individual alignments and maximum parsimony analyses of the six single genes were

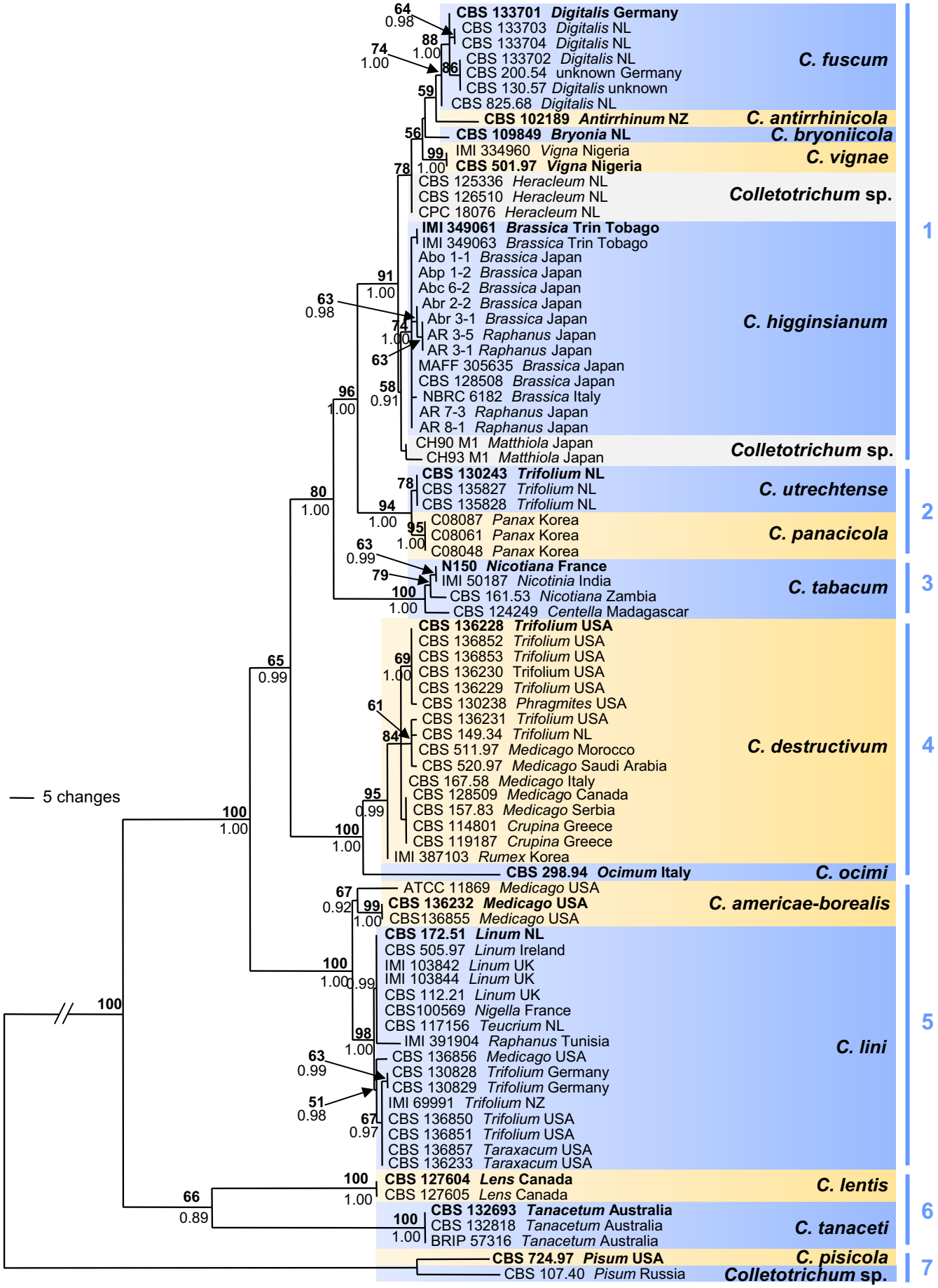

Fig. 1. The first of 14 equally most parsimonious trees obtained from a heuristic search of the combined ITS, GAPDH, CHS-1, ACT, HIS3 and TUB2 sequences alignment of the *Colletotrichum destructivum* species complex. Bootstrap support values above 50 % (bold) and Bayesian posterior probability values above 0.90 are shown at the nodes. *Colletotrichum pisicola* CBS 724.97 and *Colletotrichum* sp. CBS 107.40 are used as outgroup. Numbers of ex-holotype, ex-neotype and ex-epitype isolates are emphasised in bold. Strain numbers are followed by substrate (host genus) and country of origin, NL = Netherlands, NZ = New Zealand, Trin Tobago = Trinidad and Tobago. Main clades are indicated by blue lines. Branches that are crossed by diagonal lines are shortened by 50 %.

compared with respect to their performance in species recognition. None of the loci differentiated all clades, but TUB2 provided the highest resolution of the tested loci. All clades are recognised by using a combination of both TUB2 and GAPDH sequences; other loci only recognised some of the species. Some species differ only in one or two nucleotides (see notes accompanying each species).

Taxonomy

Based on DNA sequence data and morphology, the 83 isolates studied (Table 1) are assigned to 16 species, including eight species that are considered to be new to science. All species studied in culture are characterised below.

Colletotrichum americae-borealis Damm, **sp. nov.** MycoBank MB809398. Fig. 2.

Etymology: The species epithet is derived from the region where the species was collected, North America.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–7.5 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores formed directly on hyphae or on a cushion of pale brown, angular cells, 3–6.5 µm diam. *Setae* medium brown, smooth-walled to finely verruculose, 55–230 µm long, 1–4-septate, base cylindrical to conical, 2.5–7.5 µm diam, tip ± acute to ± rounded. *Conidiophores* hyaline to pale brown, smooth-walled, septate, branched, to 40 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical to ampulliform, sometimes intercalary (necks not separated from hyphae by septum), 9.5–24.5 × 3.5–5 µm, opening 1–1.5 µm diam, collarette 0.5–1 µm long, periclinal thickening observed. *Conidia* hyaline, smooth-walled, aseptate, cylindrical to fusoid, straight to slightly curved, both ends rounded, (13.5–)15.5–18(–19) × 3.5–4 µm, av. ± SD = 16.6 ± 1.3 × 3.7 ± 0.2 µm, L/W ratio = 4.5, conidia of strain ATCC 11869 shorter, measuring (9.5–)11.5–15.5(–17.5) × (3–)3.5–4(–4.5) µm, av. ± SD = 13.5 ± 2.2 × 3.8 ± 0.4 µm, L/W ratio = 3.5. *Appressoria* not observed, appressoria of strain CBS 136855 single or in loose groups, medium to dark brown, smooth-walled, ellipsoid, clavate or irregular outline, with an undulate to lobate margin, (4.5–)6–10.5(–13) × (3.5–)4–7(–10) µm, av. ± SD = 8.1 ± 2.2 × 5.4 ± 1.5 µm, L/W ratio = 1.5.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on pale brown, angular cells, 4–7 µm diam. *Setae* medium brown, smooth-walled, 8–250 µm long, 1–6-septate, base cylindrical to conical, 5–10 µm diam, tip ± acute to ± rounded. *Conidiophores* hyaline to pale brown, smooth-walled, simple or septate and branched, to 30 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled,

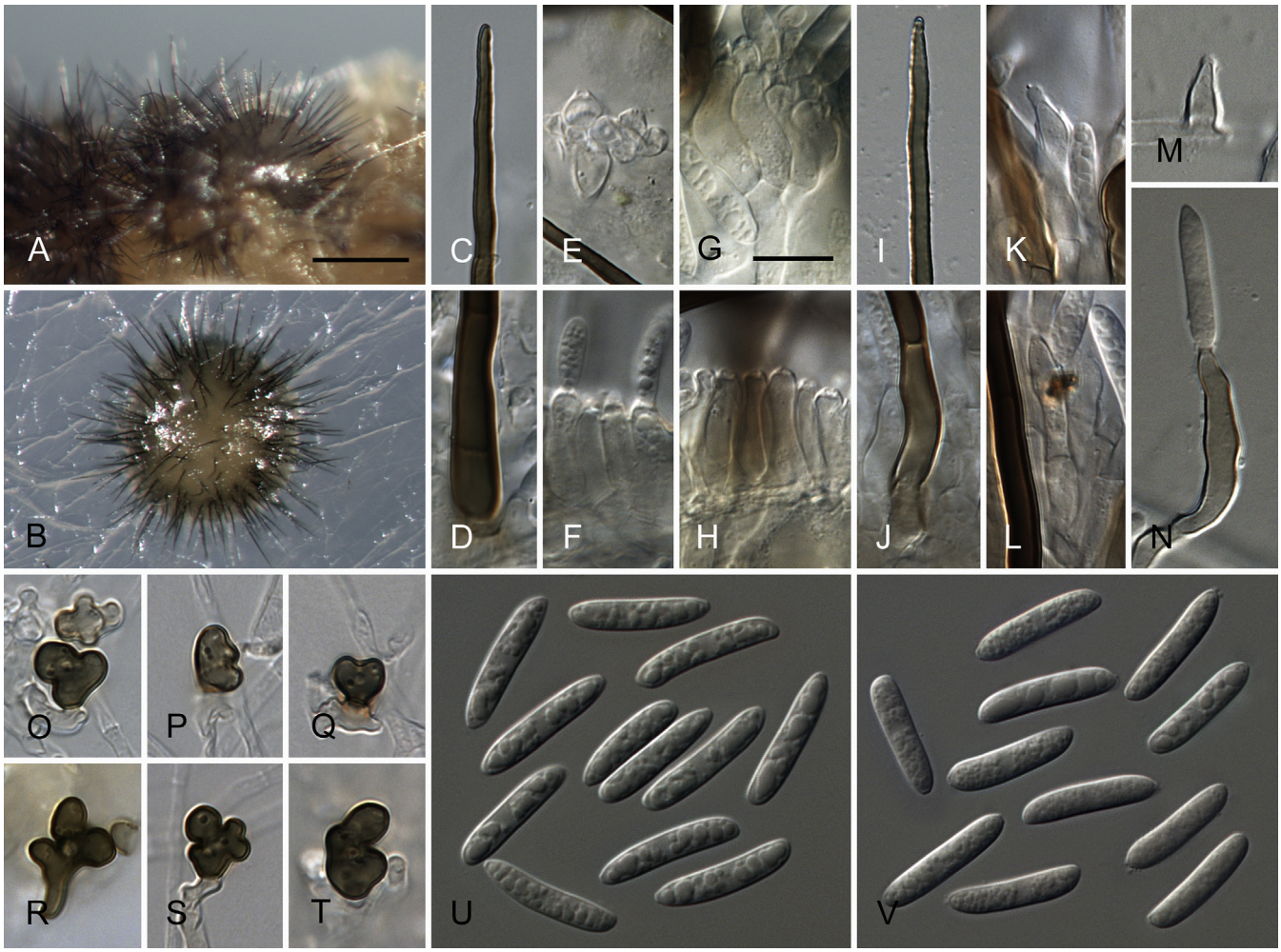

Fig. 2. *Colletotrichum americae-borealis* (A–N, U–V from ex-holotype strain CBS 136232. O–T from strain CBS 136855). A–B. Conidiomata. C, I. Tip of a seta. D, J. Base of a seta. E–H, K–N. Conidiophores. O–T. Appressoria. U–V. Conidia. A, C–H, U. from *Anthriscus* stem. B, I–T, V. from SNA. A–B. DM, C–V. DIC, Scale bars: A = 200 µm, G = 10 µm. Scale bar of A applies to A–B. Scale bar of G applies to C–V.

cylindrical to ampulliform, 8.5–19 × 3.5–5.5 µm, opening 1–2 µm diam, collarette 0.5–1 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, cylindrical to fusoid, straight to slightly curved, both ends rounded, (14.5–)16.5–18.5(–19.5) × (3–)3.5(–4) µm, av. ± SD = 17.4 ± 1.0 × 3.5 ± 0.2 µm, L/W ratio = 5.0, conidia of strain ATCC 11869 shorter, measuring (8–)13–18(–19.5) × 3–4 µm, av. ± SD = 15.5 ± 2.4 × 3.8 ± 0.3 µm, L/W ratio = 4.5.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline, pale cinnamon in the centre, agar medium, filter paper and *Anthriscus* stem partly covered with saffron to dark grey acervuli, medium and filter paper partly covered with sparse, whitish aerial mycelium, reverse same colours; growth 21.5–25 mm in 7 d (35–37 mm in 10 d). Colonies on OA flat with entire margin; buff, rosy buff to saffron, towards the centre saffron to dark grey acervuli, aerial mycelium lacking, reverse buff to rosy buff, growth 22.5–25 mm in 7 d (33.5–36 mm in 10 d). *Conidia* in mass saffron.

Materials examined: **USA**, Utah, Bluffdale (near Salt Lake City), from stems of *Medicago sativa*, 25 Aug. 2013, U. Damm (CBS H-21661 **holotype**, culture ex-holotype CBS 136232); Utah, Bluffdale (near Salt Lake City), from stems of *Medicago sativa*, 25 Aug. 2013, U. Damm, culture CBS 136855; Iowa, from *Medicago sativa*, collection date and collector unknown, (received from R. O'Connell, before from F. Uruburu, deposited in ATCC collection by L.H. Tiffany) culture ATCC 11869 = CPC 18946 = LARS 373.

Notes: The conidial shape of *C. americae-borealis* is similar to that of *C. lini*, but more complex appressoria were observed. In contrast to most of the other species in this complex, setae were very abundant. Several species have been described from *Trifolium* and *Medicago* that are discussed under *C. destructivum*.

The ITS and GAPDH sequences of *C. americae-borealis* are the same as those of *C. lini*. This species can be distinguished from other species in this complex by TUB2, CHS-1, HIS3 and ACT sequences.

Strain ATCC 11869 shows additional differences in CHS-1, HIS3 and ACT sequences to strains CBS 136232 and CBS 136855, the other two strains of this species studied. We prefer to treat this strain as *C. americae-borealis* for the present, because it has the same host and origin as the other two strains. This strain was hardly sporulating; appressoria resembled those of strains CBS 136232 and CBS 136855. Strain ATCC 11869 was deposited in the ATCC collection by L.H. Tiffany, and apparently belongs to the large collection of *Colletotrichum* isolates from legumes studied by Tiffany & Gilman (1954). It would be interesting to include more isolates related to ATCC 11869 in a future study to determine whether ATCC 11869 and additional isolates might form a distinct clade or reveal morphological or biological differences to the ex-type strain of *C. americae-borealis*.

The closest match in a blastn search with the TUB2 sequence of strain CBS 136232 was with 99 % identity (1 nucleotide difference) *C. linicola* (= *C. lini*) strain CBS 172.51 (GenBank JQ005849, O'Connell *et al.* 2012), which is included in this study. Blastn searches with the ITS sequence of strain CBS 136232 resulted in 100 % matches with sequences of *C. destructivum* (*s. lat.*) strains 1212, MP11 (GenBank KF181248, KF181247, Z. Wen & Z. Nan, an unpublished study on alfalfa root rot in Gansu, China), DAOM 179749 from an unknown host (GenBank EU400143, Chen *et al.* 2007) and strain Hamedan from clover in Iran (GenBank FJ185789, Zafari & Tarrah 2009), *C. linicola* (= *C. lini*) strain CBS 172.51 (GenBank JQ005765, O'Connell *et al.* 2012 and GenBank AB046609, Moriwaki *et al.* 2002), a *C. linicola* (= *C. lini*) isolate from *Convolvulus* in Turkey (GenBank EU000060, Tunali *et al.* 2008), unidentified fungus strains DJJ15 and DY20 from *Oxytropis* (GenBank JF461333, JF461335, J. Wang, unpubl. study), *C. higginsianum* strain IMI 391904 from *Raphanus* in Tunisia (GenBank JX499034, Naumann & Wicklow 2013) that is included in this study and re-identified as *C. lini*, *Colletotrichum* sp. isolates 2002 from *Holcus* (GenBank FN386304, Sánchez Márquez *et al.* 2012) and 842 and 865 from *Arabidopsis* (GenBank JX982460, JX982461, Garcia *et al.* 2013), both the latter reports concerning endophytes isolated in Spain.

Colletotrichum antirrhinicola Damm, **sp. nov.** MycoBank MB809399. Fig. 3.

Etymology: The species epithet is derived from its host plant *Antirrhinum*.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–10 µm diam, hyaline, some are pale to medium brown, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae. *Setae* pale to medium brown, verruculose, 30–100 µm long, 1–4-septate, base cylindrical, conical to ± inflated, 5.5–6.5 µm diam, tip rounded. *Conidiophores* hyaline, smooth-walled, septate, branched, to 35 µm long. *Conidiogenous cells* hyaline, smooth-walled, cylindrical to ampulliform, sometimes intercalary (necks not separated from hyphae by septum), polyphialides observed, 8.5–25 × 3.5–5.5 µm, opening 1.5–2 µm diam, collarette 1 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, straight to slightly curved, with one end round and the other truncate, (14.5–)15.5–19(–23.5) × (3.5–)4–4.5(–5) µm, av. ± SD = 17.2 ± 1.7 × 4.3 ± 0.3 µm, L/W ratio = 4.0. *Appressoria* single, medium brown, smooth-walled, subglobose, ovate to broadly elliptical in outline, with an entire or undulate margin, (9–)9.5–12(–13.5) × (5–)6–8(–10) µm, av. ± SD = 10.9 ± 1.3 × 7.0 ± 1.0 µm, L/W ratio = 1.5.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on pale brown, angular cells, 3–8 µm diam. *Setae* pale to medium brown, smooth-walled, 35–170 µm long, 1–3-septate, base cylindrical to conical, 5.5–6 µm diam, tip ± acute to ± rounded. *Conidiophores* hyaline, smooth-walled, septate, branched, to 25 µm long. *Conidiogenous cells* hyaline, smooth-walled, cylindrical to ampulliform, 11–15 × 4–5.5 µm, opening 1–1.5 µm diam, collarette 1 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, straight to slightly curved, with one end round and the other truncate, (13–)15.5–19(–20) × (3.5–)4–4.5(–5) µm, av. ± SD = 17.3 ± 1.6 × 4.2 ± 0.4 µm, L/W ratio = 4.1.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline to pale rosy-buff, filter paper and agar medium in centre partly grey, agar medium, filter paper and *Anthriscus* stem partly covered with white aerial mycelium, reverse same colours; growth 21.5–23 mm in 7 d (33–34.5 mm in 10 d). Colonies on OA flat with entire margin; buff, partly covered with black acervuli and salmon conidial masses, aerial mycelium lacking, reverse

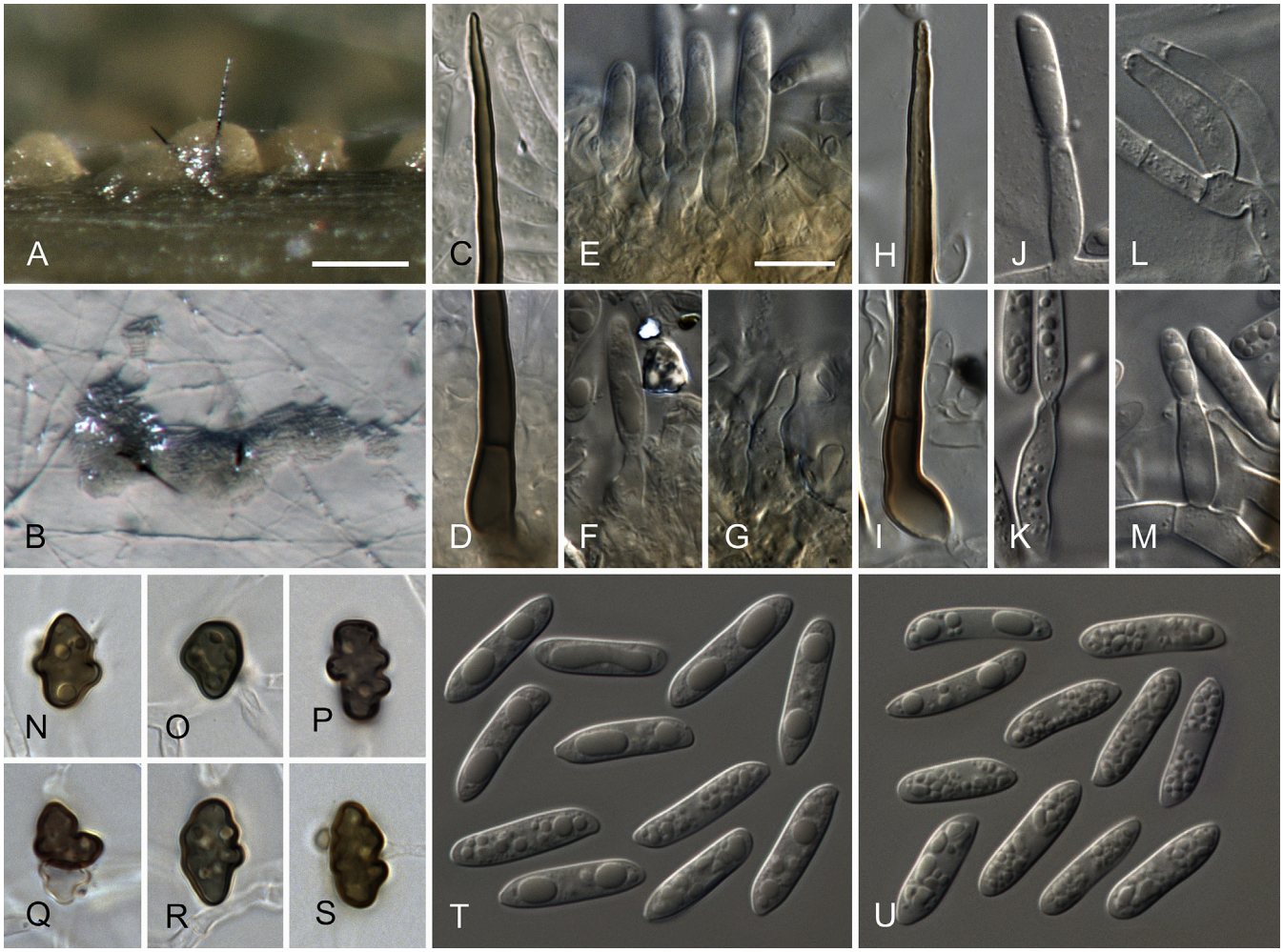

Fig. 3. *Colletotrichum antirrhinicola* (from ex-holotype strain CBS 102189). A–B. Conidiomata. C, H. Tip of a seta. D, I. Base of a seta. E–G, J–M. Conidiophores. N–S. Appressoria. T–U. Conidia. A, C–G, T. from *Anthriscus* stem. B, H–S, U. from SNA. A–B. DM, C–U. DIC, Scale bars: A = 100 µm, E = 10 µm. Scale bar of A applies to A–B. Scale bar of E applies to C–U.

buff, rosy-buff to pale olivaceous-grey, growth 20.5–22 mm in 7 d (30–31.5 mm in 10 d). *Conidia* in mass salmon.

Material examined: **New Zealand**, Auckland, Kingsland, from foliage of *Antirrhinum majus*, collection date unknown (deposited in CBS collection Sep. 1999 by C.F. Hill, isolated 22 Jul. 1999 by H.M. Dance, Agriquality N2, No. 017), HM Dance (CBS H-21647 **holotype**, culture ex-holotype CBS 102189).

Notes: Colletotrichum antirrhinicola is only known from snapdragon (*Antirrhinum majus*, *Scrophulariaceae*) in New Zealand. The species can be identified by its unique GAPDH and ITS sequences. The HIS3 sequence is the same as that of *C. fuscum*, while the ACT sequence is identical with *C. fuscum* and *C. bryoniicola*. Closest match in a blastn search with the ITS sequence of CBS 102189 with 99 % identity (1 nucleotide difference) was *C. fuscum* strain DAOM 216112 from an unknown host (GenBank EU400144, Chen *et al.* 2007), while the most similar GAPDH sequences on NCBI GenBank are 96 % identical to that of CBS 102189. In blastn searches, ACT and HIS3 sequences of CBS 102189 are identical with GenBank JQ005825 and GenBank JQ005804, respectively from *C. fuscum* CBS 130.57 (O'Connell *et al.* 2012) that are included here.

Tomioka *et al.* (2011) reported *C. destructivum* to cause a severe anthracnose disease on leaves of *A. majus* in Japan. ITS sequences of two strains (MAFF 239947, MAFF 239948) are available in NCBI GenBank (GenBank AB334521, AB334522); additionally, ACT, EF1-α, GAPDH, ITS and TUB2 sequences are available on NIAS GenBank. However, none of these sequences agree with those of strain CBS 102189 (95–99 % identity); the strains from Japan therefore probably represent a different species, most likely in the same species complex.

Stewart (1900a, b) reported a new anthracnose disease of cultivated snapdragon in the USA as *C. antirrhini*. The description by Stewart (1900b) indicates the species may belong to the *C. destructivum* complex with conidia measuring 16–21 × 4 µm. The species was regarded as synonym of *C. gloeosporioides* by von Arx (1957), and is listed as a synonym of *C. coccodes* in Index Fungorum (www.indexfungorum.org, retrieved 20 Aug. 2014). However, as there are apparently several *Colletotrichum* species causing anthracnose on this host we refrain from epitypifying this species with a strain from New Zealand instead of the USA, and rather describe it here as a new species.

Strain CBS 102189 was previously identified as *C. fuscum* by C.F. Hill. A strain from the same host and country (IMI 197877), also identified by C.F. Hill but apparently much earlier, as well as collections from UK were listed by Sutton (1980) as *C. fuscum*, which is closely related to *C. antirrhinicola*.

Colletotrichum bryoniicola Damm, **sp. nov.** MycoBank MB809400. Fig. 4.

Etymology: The species epithet is derived from its host plant *Bryonia*.

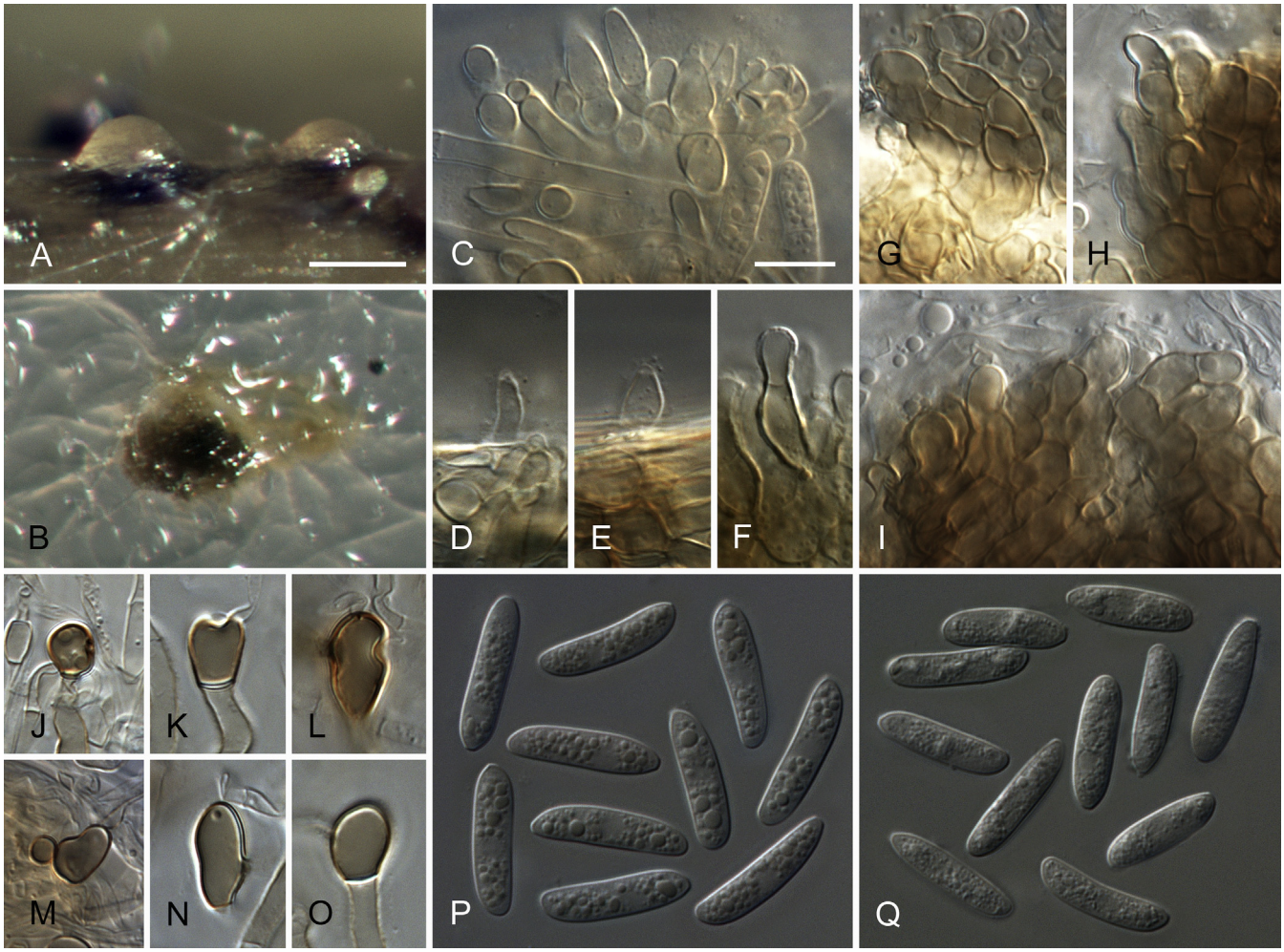

Fig. 4. *Colletotrichum bryoniicola* (from ex-holotype strain CBS 109849). A–B. Conidiomata. C–I. Conidiophores. J–O. Appressorium-like structures. P–Q. Conidia. A, C–F, P. from *Anthriscus* stem. B, G–O, Q. from SNA. A–B. DM, C–Q. DIC, Scale bars: A = 100 µm, C = 10 µm. Scale bar of A applies to A–B. Scale bar of C applies to C–Q.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–10.5 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores formed directly on hyphae. Additionally, structures formed resembling the basal cushions of acervuli, but lacking conidiophores, cells angular to roundish, pale brown, 3.5–10 µm diam. *Setae* not observed. *Conidiophores* hyaline to pale brown, smooth-walled, sometimes septate and branched, to 15 µm long. *Conidiogenous cells* rarely observed, hyaline to pale brown, smooth-walled, cylindrical to conical, 5.5–10 × 3.5–4 µm, opening 1–1.5 µm diam, collarette 0.5 µm long, periclinal thickening not observed. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, straight to slightly curved, with one end round and the other truncate, (13.5–)15–18.5(–22) × 4–5(–5.5) µm, av. ± SD = 16.8 ± 1.6 × 4.6 ± 0.4 µm, L/W ratio = 3.6. *Appressoria* not observed on the undersurface of the medium. *Appressoria-like structures* that possibly function as chlamydospores were observed within the medium. These are single or in loose groups, pale brown, smooth-walled, subglobose to elliptical in outline, with an entire or slightly undulate margin, (3.5–)4–10(–18) × (2.5–)3.5–6.5(–7.5) µm, av. ± SD = 7.1 ± 3.0 × 4.9 ± 1.5 µm, L/W ratio = 1.4.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores formed on pale brown, angular cells, 3–9 µm diam. *Setae* not observed. *Conidiophores* rarely seen, pale brown, smooth-walled. *Conidiogenous cells* hyaline to pale brown, smooth-walled, doliiform, ampulliform to cylindrical, 7–13.5 × 3–5 µm, opening 1–1.5 µm diam, collarette 0.5 µm long, periclinal thickening not observed. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, straight to slightly curved, with one end round and the other truncate, (16–)17–19.5(–21) × 4.5(–5) µm, av. ± SD = 18.2 ± 1.1 × 4.5 ± 0.1 µm, L/W ratio = 4.0.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline, agar medium, filter paper and *Anthriscus* stem partly covered with white aerial mycelium, reverse same colours; growth 20.5–21.5 mm in 7 d (29.5–31.5 mm in 10 d). Colonies on OA flat with entire margin; buff, with cinnamon, olivaceous-grey to iron-grey sectors, aerial mycelium lacking, reverse same colours, growth 17.5–18.5 mm in 7 d (27.5–29 mm in 10 d). *Conidia* in mass whitish to pale salmon.

Material examined: **Netherlands**, Wissenkerke, Camperduin, coord. 35.5/401.6, from decaying leaves of *Bryonia dioica*, 27 Aug. 2001, G. Verkley, No. V1114 (CBS H-21663 **holotype**, culture ex-holotype CBS 109849).

Notes: Colletotrichum bryoniicola differs from closely related species in ITS, GAPDH, HIS3 and TUB2 sequences by a single nucleotide in each locus. The ACT sequence is the same as that of *C. fuscum* and *C. antirrhinicola*, the CHS-1 sequence is identical with that of *C. tanaceti*. There are no previously accessioned sequences of a *Colletotrichum* species from *Bryonia* in GenBank. With the exception of the ACT and CHS-1 sequences, there are no sequences in GenBank that are identical to those of *C. bryoniicola*.

Conidia of *C. bryoniicola* are broader (≥ 4 µm on SNA, ≥ 4.5 µm on *Anthriscus* stem) than the other species in the *C. destructivum* complex, no setae were observed and the conidiogenous cells are very indistinct.

A species from *Bryonia dioica* (*Cucurbitaceae*) was previously described *ad interim* by Maire (1917), as *C. bryoniae*. Although Maire (1917) did not mention any connection of the new species from Alger (= Algiers), Mauretania (today Algeria) with *C. oligochaetum* f. *bryoniae* Ferraris from *B. dioica* in Italy (Ferraris & Massa 1912), that taxon was accepted as an independent species by Saccardo *et al.* (1931) and cited incorrectly as *C. bryoniae* (Ferraris) Maire (1917). As it is based on a *forma* of *C. oligochaetum* that was considered as a synonym of *C. orbiculare* by von Arx (1957), *C. bryoniae* was regarded as a synonym of *C. orbiculare* as well. The conidial size is similar to the strain from the Netherlands, measuring 18–22 × 4–5 µm (Maire 1917). However, as there are often several *Colletotrichum* species within this species complex causing anthracnose on the same host plants and we have no proof that this species belongs to this complex, we refrain from epitypifying this species with a strain from the Netherlands instead of Algeria, and rather describe it here as a new species. Material of the species from Algeria has not been examined and living cultures derived from its type are not available.

Colletotrichum destructivum O'Gara, Mycologia 7: 38. 1915. Fig. 5.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–9 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae or on pale to medium brown, angular cells, 3–10 µm diam, sometimes developing to dark brown, round structures on which many setae and conidiophore-like structures are formed, conidia released as well, however, most conidiophore-like structures without visible conidiogenous openings, thick-walled, septate, branched at the base, up to 70 µm long, very broad and usually broadest at the tip, cells at the tip measure 6.5–22 × 4–6 µm, surrounded by a slime sheath. *Setae* medium brown, smooth-walled to finely verruculose, sometimes verrucose, 50–180 µm long, 1–3-septate, base cylindrical, conical, sometimes ± inflated, 3.5–6 µm diam, tip ± rounded to ± acute. *Conidiophores* pale to medium brown, smooth-walled, septate, branched, to 85 µm long. *Conidiogenous cells* pale to medium brown, smooth-walled, elongate-ampulliform to cylindrical, 9.5–17 × 3.5–5 µm, opening 1–1.5 µm diam, collarette 0.5–1 µm long, periclinal thickening visible. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, straight to slightly curved, with both ends ± rounded, (14–)

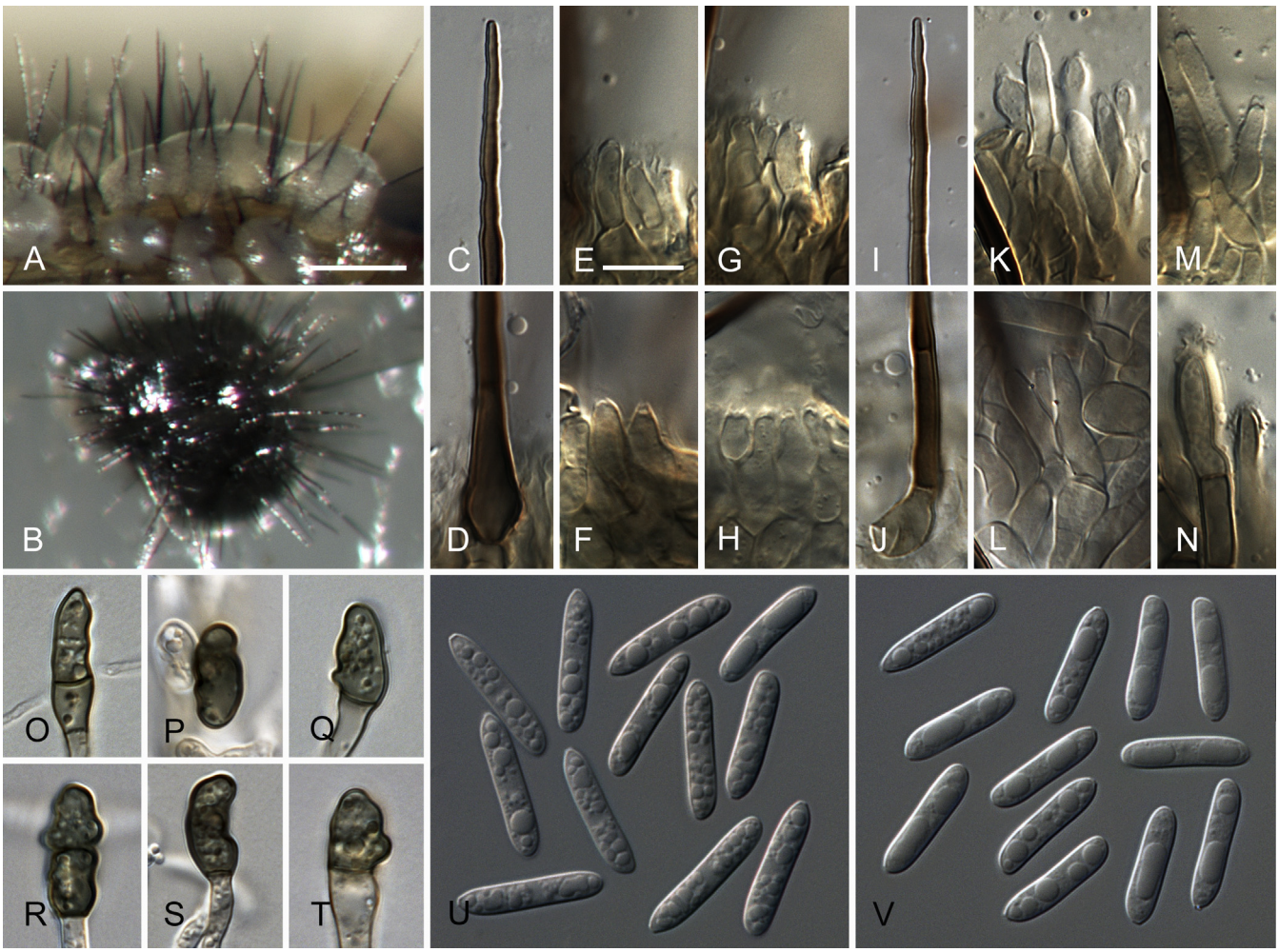

Fig. 5. *Colletotrichum destructivum* (from ex-epitype strain CBS 136228). A–B. Conidiomata. C, I. Tip of a seta. D, J. Base of a seta. E–H, K–N. Conidiophores. O–T. Appressoria. U–V. Conidia. A, C–H, U. from *Anthriscus* stem. B, I–T, V. from SNA. A–B. DM, C–V. DIC, Scale bars: A = 100 µm, E = 10 µm. Scale bar of A applies to A–B. Scale bar of E applies to C–V.

14.5–16.5(–18) × 3.5–4(–4.5) µm, av. ± SD = 15.4 ± 0.8 × 3.7 ± 0.2 µm, L/W ratio = 4.2. *Appressoria* single, pale brown, smooth-walled, clavate, fusiform to ellipsoidal outline, with a lobate, undulate or crenate margin, (6.5–)10–15.5(–20.5) × (4.5–)5–8(–10.5) µm, av. ± SD = 12.5 ± 2.7 × 6.7 ± 1.5 µm, L/W ratio = 1.9, Appressoria of strain CBS 149.34 smaller, measuring (4–)6–14(–25) × (3.5–)4.5–7.5(–10) µm, av. ± SD = 9.8 ± 4.1 × 5.9 ± 1.5 µm, L/W ratio = 1.7, strain CBS 149.34 also forms appressorium-like structures inside the medium, single, medium brown, smooth-walled, subglobose, ovate to broadly elliptical in outline, with an entire or undulate margin, (3.5–)5–10(–13) × (3–)3.5–7(–8.5) µm, av. ± SD = 7.6 ± 2.5 × 5.2 ± 1.7 µm, L/W ratio = 1.4.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on a cushion of pale to medium brown, angular cells, 3–8.5 µm diam that are intermingled and surrounded by medium brown, thick-walled hyphae, with ± inflated cells, up to 8.5 µm diam. *Setae* medium brown, smooth-walled, towards the tip often verruculose to verrucose, constricted and slightly wavy, 65–110 µm long, 1–4-septate, base conical to ± inflated, 4.5–12 µm diam, tip ± rounded to ± acute. *Conidiophores* hyaline to pale brown, smooth-walled, simple or septate and branched, to 25 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical, doliiform to ampulliform, 5–14 × 2.5–5.5 µm, opening 1–2 µm diam, collarette 0.5–1.5 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, straight to slightly curved, with both ends ± rounded, (15–)16–18(–19) × (3–)3.5–4 µm, av. ± SD = 16.9 ± 1.0 × 3.6 ± 0.2 µm, L/W ratio = 4.7.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline to honey, agar medium and filter paper partly covered by sparse filty white aerial mycelium; agar medium, filter paper and *Anthriscus* stem partly covered with grey to black acervuli, reverse same colours; growth 23–25 mm in 7 d (36–37.5 mm in 10 d). Colonies on OA flat with entire margin; buff to honey, almost entirely covered with grey to black acervuli; partly covered with whitish to grey aerial mycelium, reverse buff to olivaceous-grey, growth 25–27.5 mm in 7 d (37.5–40 mm in 10 d). *Conidia* in mass whitish to rosy-buff.

Materials examined: **Canada**, south-western Ontario, from anthracnose on stems of *Medicago sativa*, 1985/86 collector unknown (deposited by R. O'Connell, before from G.J. Boland, A 22), culture CBS 128509 = LARS 320. **Korea**, from *Rumex* sp., collection date and collector unknown (CBS H-21655, culture ex IMI 387103 = CPC 18082). **Netherlands**, experimental plot of P.M.L.Tammes, from stem burn of *Trifolium* sp., 1 Oct. 1934, P.M.L.Tammes, culture CBS 149.34. **USA**, Utah, probably Salt Lake City, on stems and leaves of *Trifolium pratense*, 30 Jun. 1914, P. J. O'Gara (BPI 397373 **holotype**); Utah, Salt Lake City, cemetery, from small black spots on petioles of *T. hybridum*, 24 Aug. 2013, U. Damm (CBS H-21652 **epitype, here designated**, MBT178515, culture ex-epitype CBS 136228); Utah, Syracuse (close to Salt Lake City), pasture, from small black spots on petioles of *T. hybridum*, 24 Aug. 2013, U. Damm, CBS H-21653, culture CBS 136229; from *Phragmites* sp., collection date and collector unknown, CBS H-21654, culture CBS 130238.

Notes: Colletotrichum destructivum was described by O'Gara (1915) from stems and petioles of red clover (*Trifolium pratense*) and alsike clover (*T. hybridum*) in clover fields in the Salt Lake Valley, Utah, USA. The species forms minute acervuli, 25–70 µm diam, hyaline conidia with 1–4 guttules that are straight to slightly curved with rounded apices and bases, measuring 14–22 × 3.5–5 µm, few to numerous setae that are straight, curved to flexuous, often nodulose, aseptate to obscure 1-septate, subacute to rounded, constricted at the apex, 38–205 µm long and 4.5–7 µm diam at the basis (O'Gara 1915). Conidia from small acervuli were observed on stems, petioles and leaves of the holotype specimen (BPI 397373) and measure (14–)15–19.5(–23.5) × (3–)4–4.5 µm, av. ± SD = 17.2 ± 2.1 × 3.8 ± 0.4 µm, L/W ratio = 4.5; setae were 50–110 µm long with a cylindrical to ± inflated base, 3.5–8 µm diam and a ± rounded tip.

The specimen BPI 397373 was collected on 30 June 1914 by P.J. O'Gara and designated no. 20. The package has a type stamp and origins from Fungi Utahensis, Herbarium of Department of Agricultural Investigation, American Smelting & Refining Co., Salt Lake City, Utah, which was the institute where P.J. O'Gara used to work as stated in the publication. There is an isotype of this fungus in herbarium NY with the information "Incorporated herbarium: Garrett Herbarium, University of Utah". There is no specimen of *C. destructivum* available in the Garrett Herbarium any more; the specimen was apparently sent away together with many specimens from that herbarium (M. Power, in lit.). This agrees with a note on the specimen packet stating "Rec. by Path. Coll. Apr. 8, 1921"; BPI 397373 must therefore be the holotype.

In order to epitypify *C. destructivum*, collections of *Trifolium hybridum* and *Medicago sativa* were made in August 2014 from field as well as urban locations in and around Salt Lake City; *T. pratense* was not found in the area. *Colletotrichum* spp. were isolated from small black spots on stems, petioles and leaves of both host plants. Isolates of the *C. destructivum* species complex were identified based on morphology. Some of the isolates grouped with a species that had often been collected from both host genera worldwide, for which the name *C. destructivum* is usually applied. The other isolates belonged to *C. lini* or a species closely related to *C. lini*. *Colletotrichum lini* contained both *Trifolium* and *Medicago* isolates, the other clade only *Medicago* isolates (see *C. americae-borealis*). It is possible that O'Gara (1915) collected more than one species as well. However, there is not enough material available of the holotype to extract DNA for molecular examination. The two species collected from clover are morphologically very similar. However, setae of the ex-epitype strain CBS 136228 grown on *Anthriscus* stem were often constricted towards the tip, which is in accordance with the observations made by O'Gara (1915). This was not observed in *C. lini*.

Several *Colletotrichum* and *Gloeosporium* species have been described from *Trifolium* and *Medicago* as already discussed in Damm *et al.* (2013). Except for *Gm. trifolii* and *Gm. medicaginis*, these species were described later than *C. destructivum* and cannot be considered as possible synonyms of *C. destructivum*. Except for *C. destructivum* and *C. trifolii*, these names have not been used since their description. *Colletotrichum trifolii* was originally described from *T. pratense* in the USA (Bain & Essary 1906); the species was epitypified recently and revealed to belong to the *C. orbiculare* species complex (Damm *et al.* 2013) that is distinct from the species complex studied here (Cannon *et al.* 2012). Strain CBS 149.34 was previously identified as *C. trifolii* (Nirenberg *et al.* 2002), but re-identified here as *C. destructivum*.

Gloeosporium trifolii described from *T. pratense* in Albany, NY, USA (Peck 1879, publ. 1883) forms conidia that measure 15–23 × 4–6.3 µm, which are slightly larger than *C. destructivum*. *Gloeosporium medicaginis* forms acervuli on *Medicago sativa* in Kansas, USA, with cylindrical conidia that are

subhyaline and mostly narrowed in the middle, measuring 15–20 × 3–4 µm (Ellis & Kellerman 1887). Conidia that are constricted in the middle have not been observed in this species complex. We have not studied the type specimens of these species. However, even if either of these "forgotten" species belong to the *C. destructivum* species complex, it would be difficult to link recent collections to one of them based on morphology alone.

Colletotrichum sativum, a species described from *Vicia sativa* in Louisiana, USA (Horn 1952), was listed as a synonym of *C. destructivum* by von Arx (1957). We cannot confirm this synonymy as no strain from *Vicia sativa* belonging to this species complex was studied.

The description and drawing of *C. rumicis-crispi* from *Rumex crispus* in Taiwan described by Sawada (1959) are similar to our observations made of *C. destructivum* that includes a strain from *Rumex* in Korea (IMI 387103). *Colletotrichum rumicis-crispi* is probably a synonym of *C. destructivum*. However, as we have not seen the type specimen, we cannot confirm this.

Strain IMI 387103 differs from the other *C. destructivum* sequences in ACT and TUB2 sequences in one nucleotide each, but the other loci are identical. In contrast, the ITS sequence of *C. destructivum* strain RGT-S12 from *R. gmelinii* from China (GenBank HQ674658, Hu *et al.* 2012) was 99 % identical (2 nucleotides different) with the ITS sequence of the ex-type strain of *C. destructivum* (CBS 136228), and strain IMI 387103 from *Rumex*, but identical with those of *C. higginsianum*. This indicates the occurrence of at least two *Colletotrichum* species on *Rumex*.

Manandhar *et al.* (1986) claimed *C. destructivum* to be the asexual morph of *Ga. glycines* based on morphological comparison of isolates from *Glycine* that formed a sexual morph resembling *Ga. glycines* (Lehman & Wolf 1926) and an isolate from *Medicago* that was initially identified as *C. destructivum*. The authors apparently assumed that *Colletotrichum* strains from legumes are all *C. destructivum* unless the conidia are curved. Isolates from both hosts that were included in the study of Manandhar *et al.* (1986) were sequenced and confirmed to belong to the same species that is, however, not closely related to *C. destructivum* and belongs in a different species complex (U. Damm, unpubl. data). Consequently, there is no evidence that *C. destructivum* forms a sexual morph.

The hemibiotrophic infection of *Medicago sativa* by *C. destructivum* was observed by Latunde-Dada *et al.* (1997). The fungus initially produced large, prominently multilobed infection structures that were localised within single epidermal cells of the infected host. Two of the three isolates studied, LARS 202 (= CBS 511.97) and LARS 709 (= CBS 520.97), were confirmed as *C. destructivum s. str.* in this study. The third isolate LARS 319 originated from the same collection (A22) from Canada as *C. destructivum s. str.* strain LARS 320 (= CBS 128509), and is also included here. All isolates originated from a pathogenicity study by Boland & Brochu (1989).

Conidia of *C. destructivum* are very slightly curved and appear almost straight, similar to those of *C. tabacum*, especially on SNA; however, no dark halo around the penetration pores of appressoria was observed.

Colletotrichum destructivum can be distinguished by its ITS, HIS3, ACT and TUB2 sequences, while the GAPDH sequence is identical to that of *C. ocimi* that is described as a new species in this study. Further intraspecific grouping was observed with sequences of all loci studied. Strain IMI 387103 from *Rumex* in Korea was the most distant strain from the rest of the *C. destructivum* strains that form a cluster with a bootstrap support value of 84 %. However, we refrained from considering strain IMI 387103 as a separate species as it forms a strong cluster with *C. destructivum* (95/0.99) and only differs in a few nucleotides from the majority of the *C. destructivum* strains. In order to investigate if this represents a distinct species, additional collections from *Rumex* should be studied. The three subclades with a bootstrap support $\leq$69 % did not show clear host preferences and did not suggest any further splitting of the species.

ITS sequences of a large number of isolates detected in blastn searches were identical to the ITS sequence of the ex-epitype strain (CBS 136228) of *C. destructivum*: *C. destructivum* strains CBS 149.34 from *Trifolium* in the Netherlands (GenBank JQ005764, O'Connell *et al.* 2012) that is included in this study, Coll-48, Coll-68, Coll-75 and CC 657 from *Medicago* in Serbia (GenBank JX908362, JX908363, JX908361, Vasić, unpubl. data), MAFF 239947 and MAFF 239948 from *Antirrhinum* in Japan (GenBank AB334521, AB334522, Tomioka *et al.* 2011), MAFF 410037 from *Robinia* in Japan (GenBank AB105961, Moriwaki *et al.* 2002), CGMCC 3.15129 from *Bletilla* in China (GenBank JX625174, Tao *et al.* 2013), uncultured fungus clone CMH309 from house dust in the USA (GenBank KF800400, Rittenour *et al.* 2013), DAOM 196849 from an unknown host (GenBank EU400156, Chen *et al.* 2007), *C. trifolii* isolate UQ349 from *Medicago* in Australia (GenBank AF451909, Ford *et al.* 2004) and CBS 149.34 (GenBank AJ301942, Nirenberg *et al.* 2002) and *C. cf. gloeosporioides* strain AR 4031 (= CBS 119187) from *Crupina* in Greece (GenBank AY539806, Berner *et al.* 2004). *Colletotrichum trifolii* strain CBS 149.34 and *C. cf. gloeosporioides* CBS 119187 are included in this study and re-identified as *C. destructivum s. str.*

The TUB2 sequence of CBS 136228 is identical with GenBank JQ005848 from *C. destructivum* strain CBS 149.43 (O'Connell *et al.* 2012), 99 % (1 nucleotide difference) identical with GenBank JX625198 and JX625200 from isolates CGMCC 3.15127 and CGMCC 3.15128 and 99 % identical (3 nucleotides difference) with GenBank JX625203 from isolate CGMCC 3.15129 from *Bletilla* in China (Tao *et al.* 2013).

Colletotrichum fuscum Laubert, Gartenwelt 31: 675. 1927. Fig. 6.

= *Colletotrichum digitalis* Unamuno, Revista Real Acad. Ci. Madrid. 30: 503. 1933 – Nom. illegit., Art. 53.1

≡ *Colletotrichum unamunoi* Cash, Syll. fung. (Abellini) 26: 1222. 1972.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–9.5 µm diam, hyaline to pale brown, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores aggregated directly on pale to medium brown hyphae or on clusters of irregularly arranged medium brown hyphae. *Setae* (one observed) medium brown, smooth-walled, 71 µm long, 3-septate, base conical, 5.5 µm diam, tip rounded. *Conidiophores* hyaline to medium brown, smooth-walled, simple or septate and branched, to 20 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, ampulliform, doliiform to cylindrical, 7–18.5 × 3.5–6 µm, sometimes not separated from hyphae by a septum (intercalary) or opening with collarette formed directly on hyphae, opening 1–1.5 µm diam, collarette 0.5–1 µm long, periclinal thickening visible. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, slightly curved to straight, with one end round and the other truncate, (16–)16.5–20(–34) × (3.5–)4–4.5(–5.5) µm,

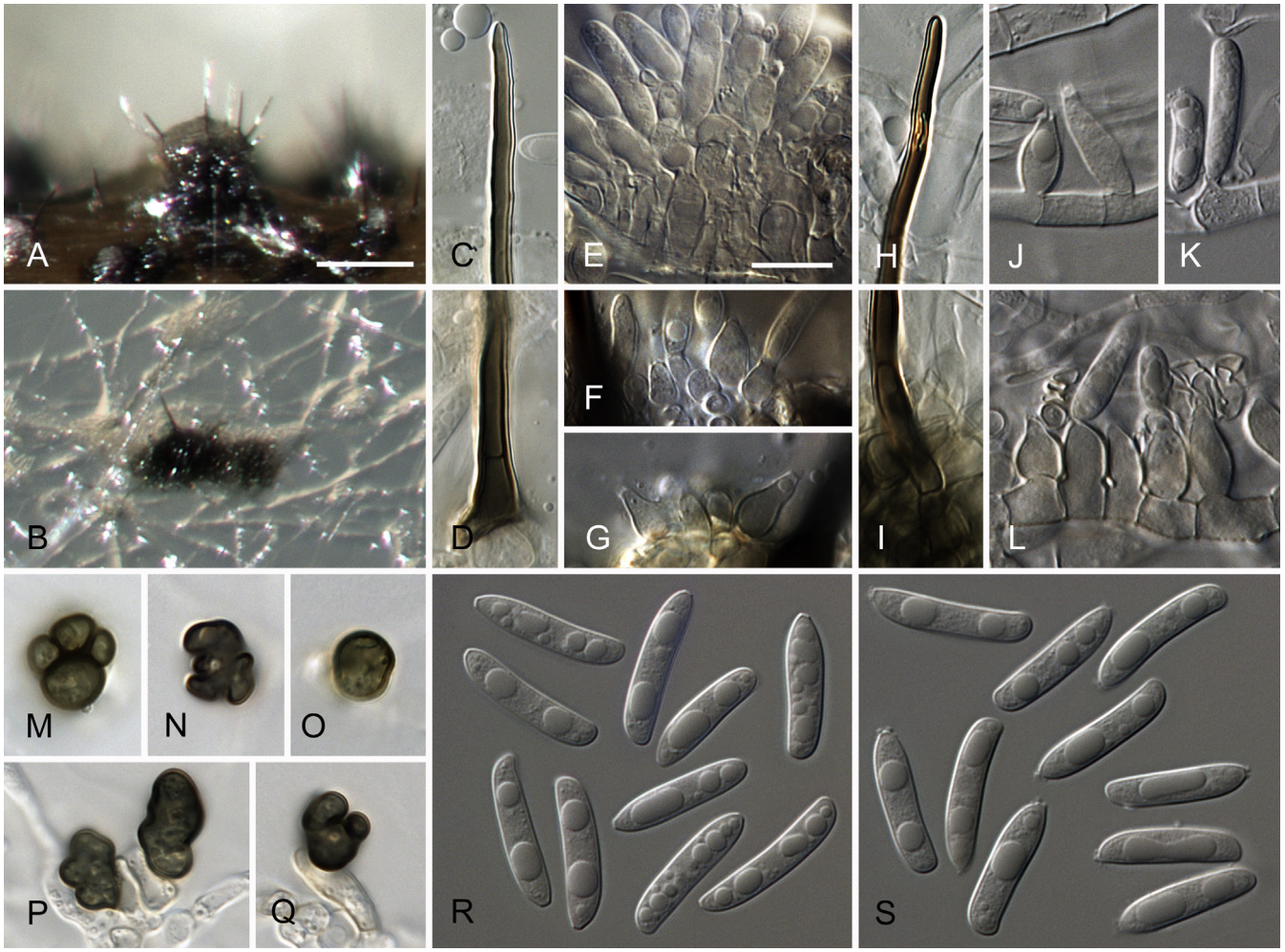

Fig. 6. *Colletotrichum fuscum* (from ex-epitype strain CBS 133701). A–B. Conidiomata. C, H. Tip of a seta. D, I. Base of a seta. E–G, J–L. Conidiophores. M–Q. Appressoria. R–S. Conidia. A, C–G, R. from *Anthriscus* stem. B, H–Q, S. from SNA. A–B. DM, C–S. DIC, Scale bars: A = 100 µm, E = 10 µm. Scale bar of A applies to A–B. Scale bar of E applies to C–S.

av. ± SD = 18.3 ± 1.9 × 4.1 ± 0.3 µm, L/W ratio = 4.5. *Appressoria* single, medium brown, smooth-walled, roundish, ellipsoidal to clavate in outline, with an lobate (to undulate) margin, (6–)8.5–14.5(–19) × (6.5–)7–10(–11.5) µm, av. ± SD = 11.5 ± 2.8 × 8.6 ± 1.5 µm, L/W ratio = 1.3.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on a cushion of medium brown, angular to roundish cells, 4.5–12.5 µm diam. *Setae* medium to dark brown, smooth-walled, 3–160 µm long, 1–3-septate, base conical, 5–7.5 µm diam, tip ± rounded to ± acute. *Conidiophores* hyaline to medium brown, smooth-walled, simple or septate and branched, to 20 µm long. *Conidiogenous cells* hyaline to medium brown, smooth-walled, ampulliform to conical, 5.5–17.5 × 3–5.5 µm, opening 1–1.5 µm diam, collarette 0.5–1 µm long, periclinal thickening visible. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, slightly curved to straight, with one end round and the other truncate, (16–)17–19.5(–20.5) × (3.5–)4–4.5(–5) µm, av. ± SD = 18.1 ± 1.4 × 4.1 ± 0.3 µm, L/W ratio = 4.4.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline to pale honey, agar medium, filter paper and *Anthriscus* stem partly covered with appressed whitish aerial mycelium, *Anthriscus* stem partly covered with black acervuli, reverse same colours; growth 21–24 mm in 7 d (35–40 mm in 10 d). Colonies on OA flat with entire margin; buff to rosy-buff with small black spots (not clearly recognisable as conidiomata) towards the centre, aerial mycelium lacking, reverse buff to rosy-buff, vinaceous buff towards the centre, growth 21–24 mm in 7 d (31–34 mm in 10 d). *Conidia* in mass rosy-buff to pale salmon.

Materials examined: **Germany**, Berlin, Zehlendorf, garden, from leaves of *Digitalis purpurea*, 1927, R. Laubert [B 70 0021851 (ex BBA acc. 9.1.1980) **lectotype, here designated,** MBT178720]; Berlin, Zehlendorf, garden, from leaves of *Digitalis purpurea*, 1927–1933, R. Laubert (B 70 0021852); Berlin, garden, from leaf of *Digitalis lutea*, 2 Aug. 2012, U. Damm (CBS H-21651 **epitype, here designated**, MBT178517, culture ex-epitype CBS 133701). **Netherlands**, Utrecht, garden, from leaf of *Digitalis obscura*, 29 Aug. 2012, U. Damm, culture CBS 133703; Baarn, garden Eemnesserweg 90, from living leaves of *Digitalis purpurea*, Nov. 1968, H.A. van der Aa, 944, CBS H-10616, culture CBS 825.68; Utrecht, from dead stem of *Heracleum* sp., 12 Aug. 2009, U. Damm, CBS H-21666, culture CBS 125336; Utrecht, from dead stem of *Heracleum* sp., 12 Aug. 2009, U. Damm, CBS H-20404, culture CBS 126510; Utrecht, from dead stem of *Heracleum* sp., 12 Aug. 2009, U. Damm, CBS H-20405, culture CPC 18076.

Notes: Colletotrichum fuscum causes anthracnose on some *Digitalis* spp. (foxglove) and was reported from the USA (Connecticut, Maryland, Oregon, Pennsylvania, South Dakota), Poland, Australia, Canada, Germany, England, New Zealand, Portugal and Czechoslovakia (Farr & Rossman 2014). According to Sutton (1980), *C. fuscum* also attacks *Antirrhinum majus* in New Zealand and the UK. However, the IMI strain from *Antirrhinum majus* in New Zealand listed by Sutton (IMI 197877) belongs to a different species (see *C. antirrhinicola*). Tomioka

et al. (2001) showed *C. fuscum* caused anthracnose of *Nemesia strumosa* in Japan. Based on morphology (and host), this Japanese collection may belong to the *C. destructivum* complex, but its identification needs to be confirmed based on molecular data. Thomas (1951) reports serious damage of *Digitalis lanata* in commercial plantings by *C. fuscum*.

Laubert (1927) described *C. fuscum* from diseased leaves of *Digitalis purpurea* in Berlin with conidia that are 12–24 µm long and 2–4 µm wide, straight or slightly clavate and then slightly curved at the narrow end, formed from short crowded conidiophores, setae 8–10 × 45–100 µm with a slightly inflated base up to 9 µm diam. Two authentic specimens were located in the fungarium B, both without type designation, of which B 70 0021851 collected by R. Laubert in 1927, was selected as lectotype of *C. fuscum*. The collection date of the second specimen (B 70 0021852) was imprecise (1927–1933); the specimen might have been collected after the publication of Laubert's description. Conidia observed on the lectotype specimen measured (15–)17–21.5(–23) × 3.5–5(–5.5) µm, av. ± SD = 19.3 ± 2.2 × 4.2 ± 0.6 µm, L/W ratio = 4.6 and resembled those seen in culture.

Several other species have been described on *Digitalis*. *Gloeosporium digitalis*, which was described from leaves of *Digitalis purpurea* in Landbohøjskolens Have, Frederiksberg, Denmark, forms smaller conidia than *C. fuscum*, measuring 8–10 × 3–4 µm and apparently lacks setae (Rostrup 1899). Goto (1938) concluded *Gm. digitalis* to be a different species. Von Arx (1957) regarded *Gm. digitalis* as a synonym of *Ascochyta digitalis* Fuckel.

However, Moesz (1931) combined *Gm. digitalis* in *Colletotrichum* on the basis of observations of a fungus on *Digitalis ferruginea* from Hungary that more closely resembled *C. fuscum* than *Gm. digitalis*. Goto (1938) regarded this fungus as a form of *C. fuscum*, while von Arx (1957) listed this species as a synonym of *C. fuscum* and called it *C. digitalis* Moesz. *Colletotrichum digitalis* could also be a different species based on shape and size of the conidia (cylindrical with blunt ends, measuring 10–15 × 3 µm) and the long conidiophores that are illustrated (Moesz 1931).

Unamuno (1933) described a *Colletotrichum* species from leaves of *Digitalis purpurea* in Spain and, apparently unaware of Moesz's combination, called it *C. digitalis* Unamuno. As this name is illegitimate (Art. 53.1), Trotter & Cash (1972) gave the species a new name, *C. unamunoi* Cash. Based on the morphological features (conidia 16–22 × 3–3.5 µm, hyaline, cylindrical, usually straight, sometimes slightly curved, rounded at both ends, setae 63 × 3.5–4 µm, brown, septate, straight, curved or flexuous, often nodular; Trotter & Cash 1972), both Goto (1938) and von Arx (1957) regarded *C. digitalis* Unamuno as a synonym of *C. fuscum*. We have not seen the type of *C. digitalis* Unamuno, but agree that this species is most likely a synonym of *C. fuscum* that seems to be the common anthracnose pathogen of several *Digitalis* spp., at least in Europe.

Colletotrichum dematium was reported from *Digitalis atropurpurea* in UK and Scotland (Kirk & Spooner 1984). We do not know whether this report refers to *C. dematium s. str.*; all species called *C. dematium* (*s. lat.*) usually have distinctly curved conidia and are not closely related with *C. fuscum* (Damm *et al.* 2009, Cannon *et al.* 2012).

Goodman (1960) discovered the phytotoxin colletotin in three strains of *C. fuscum*, one of which was obtained from the CBS collection and called the "von Arx strain". This strain is probably identical to strain CBS 130.57 that was deposited in the CBS collection in Sep. 1957 by von Arx, listed as forming colletotin with a reference to R.N. Goodman in the CBS strain database, and is included in this study.

Moriwaki *et al.* (2002) noticed the similarity and close relationship of *C. fuscum* to *C. destructivum* and assumed them to be conspecific. Preliminary multilocus phylogenies (O'Connell *et al.* 2012, Cannon *et al.* 2012) recently indicated *C. fuscum* to be a distinct species, which is confirmed in this study.

The complex appressoria and the conidiogenous cells that are often ampulliform on the two media tested are diagnostic for this species. *Colletotrichum fuscum* is distinguishable by GAPDH, but has only one nucleotide difference from *C. bryoniicola*. The ITS sequence is variable; isolates do cluster, but one strain (CBS 825.68) sits separately. The CHS-1 sequence is the same as that of *C. antirrhinicola*, the ACT sequence the same as that of *C. antirrhinicola* and *C. bryoniicola*. Additionally, unnamed isolates from *Heracleum* are basal to *C. fuscum*, *C. antirrhinicola*, *C. bryoniicola* and *C. vignae* in our phylogeny, and could represent an additional, currently unidentified species (Fig. 1).

The closest matches with the GAPDH sequence of strain CBS 133701, with 98 % identity (3 nucleotides different), are GenBank GU935850 and GU935851 from *C. higginsianum* isolates C97027 and C97031 (Choi *et al.* 2011). The closest matches with the ITS sequence, with 99 % identity (2 nucleotides different) were *C. fuscum* strains CBS 130.57 from *Digitalis* (GenBank JQ005762, O'Connell *et al.* 2012), DAOM 216112 (GenBank EU400144, Chen *et al.* 2007) and BBA 70535 from *Digitalis* in Germany (GenBank AJ301938, Nirenberg *et al.* 2002).

Colletotrichum higginsianum Sacc., J. Agric. Res., Washington 10: 161. 1917. Fig. 7.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–8.5 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* conidiophores and setae on pale brown, angular cells, 3–9 µm diam. *Setae* medium brown, smooth-walled to finely verruculose, 60–185 µm long, 1–5-septate, base cylindrical to conical, 3.5–6 µm diam, tip rounded to ± acute. *Conidiophores* hyaline, smooth-walled, septate, branched, to 35 µm long. *Conidiogenous cells* hyaline, smooth-walled, cylindrical, 8–27 × 3.5–4.5 µm, sometimes intercalary (necks not separated from hyphae by septum), opening 1–2 µm diam, collarette 1–2 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, straight to very slightly curved, with one end rounded and the other truncate, (17–)19–20.5(–21) × (3–)3.5–4(–4.5) µm, av. ± SD = 19.6 ± 0.9 × 3.7 ± 0.2 µm, L/W ratio = 5.3; conidia of strain IMI 349063 shorter, measuring (13.5–)15–19(–21.5) × 3.5–4(–4.5) µm, av. ± SD = 17.0 ± 1.8 × 3.7 ± 0.3 µm, L/W ratio = 4.6.

Appressoria in loose groups, medium brown, smooth-walled, fusiform, clavate, elliptical or irregular outline, with an entire, crenate or lobate margin, (5.5–)10–20(–28.5) × (3.5–)5–9(–12) µm, av. ± SD = 15.0 ± 5.1 × 6.8 ± 2.0 µm, L/W ratio = 2.2; appressoria of strain MAFF 305635 smaller, measuring (7.5–)9–13(–15) × (3.5–)4.5–6.5(–8) µm, av. ± SD = 11.0 ± 1.9 × 5.4 ± 0.9 µm, L/W ratio = 2.0.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on pale brown, angular cells,

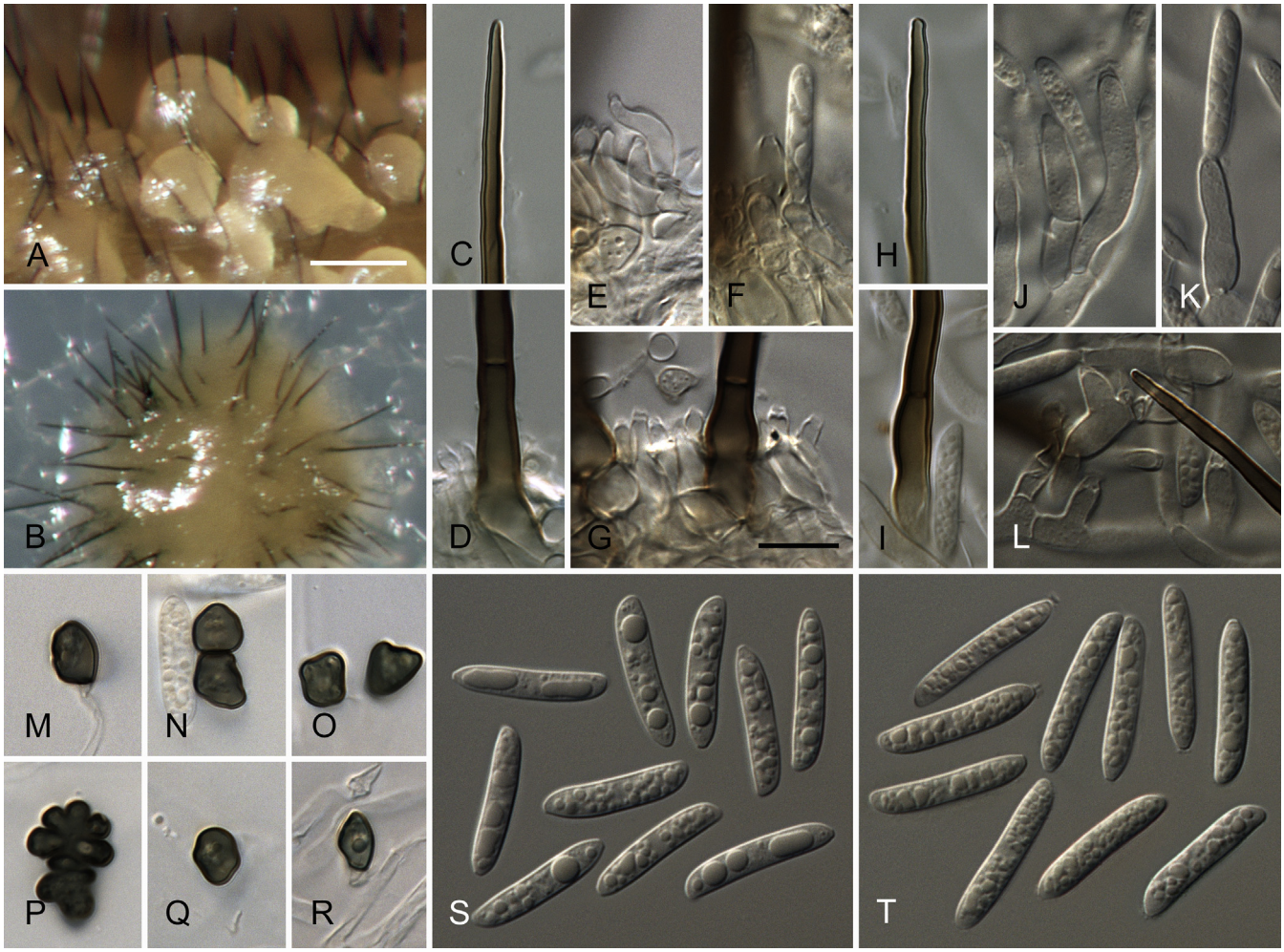

Fig. 7. *Colletotrichum higginsianum* (from ex-epitype strain IMI 349061). A–B. Conidiomata. C, H. Tip of a seta. D, I. Base of a seta. E–F, J–L. Conidiophores. G. Bases of a seta and conidiophores. M–R. Appressoria. S–T. Conidia. A, C–G, S. from *Anthriscus* stem. B, H–R, T. from SNA. A–B. DM, C–T. DIC, Scale bars: A = 100 µm, G = 10 µm. Scale bar of A applies to A–B. Scale bar of G applies to C–T.

3–7.5 µm diam. *Setae* medium brown, smooth-walled, 50–170 µm long, 2–5-septate, base cylindrical to conical, 5–12 µm diam, tip ± rounded to ± acute. *Conidiophores* hyaline to pale brown, smooth-walled, simple or septate and branched, to 15 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical to ampulliform, 8–14 × 3–3.5 µm, opening 1–1.5 µm diam, collarette 0.5–1 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, straight to very slightly curved, with one end rounded and the other truncate, (17.5–)18–20(–22) × (3–)3.5–4 µm, av. ± SD = 19.0 ± 0.9 × 3.6 ± 0.2 µm, L/W ratio = 5.2; conidia of strain IMI 349063 shorter, measuring (12.5–)15–18(–18.5) × 3.5–4.5 µm, av. ± SD = 16.5 ± 1.7 × 4.0 ± 0.3 µm, L/W ratio = 4.1.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline, agar medium, filter paper and *Anthriscus* stem partly covered with salmon to grey acervuli and floccose white aerial mycelium, filter paper partly pale luteous to pale orange, reverse same colours; growth 23–24 mm in 7 d (35–37.5 mm in 10 d); with strain IMI 349063 aerial mycelium lacking and filter paper partly pale luteous. Colonies on OA flat with entire margin; buff, saffron to pale orange, partly covered with pale orange, grey to black acervuli and floccose white aerial mycelium, reverse buff, salmon to olivaceous-grey, growth 23.5–29 mm in 7 d (36–39.5 mm in 10 d); with strain IMI 349063 aerial mycelium lacking and slower growth 20.5–22.5 mm in 7 d (30–33.5 mm in 10 d). *Conidia* in mass saffron to orange.

Materials examined: **Japan**, Edogawa, Tokyo, from *Brassica rapa* var. *komatsuna*, collection date and collector unknown (isolated 6 Oct. 1980 by H. Horie), culture MAFF 305635 = Abr 1-5 = CPC 19366, CPC 18943; Edogawa, Tokyo, from *Raphanus sativus*, collection date and collector unknown (isolated 21 Oct. 1980), culture AR 3-1 = CPC 19394; Tateyama, Chiba, from *Matthiola incana*, collection date and collector unknown (isolated Oct. 1990), CBS H-21665, culture CH90-M1 = CPC 19361; Chikura, Chiba from *Matthiola incana*, collection date and collector unknown (isolated Oct. 1990), culture CH93-M1 = CPC 19362. **Romania**, Târgu Neamț, garden near Văratec, on leaves of *Matthiola incana*, 20 Jul. 1952, C. Sandu-Ville, (GLM-F102751 **holotype of *C. mathiolae*** Sandu ex Herbarul Micologic "C. Sandu-Ville"). **Trinidad and Tobago**, Trinidad, Wallerfield, from leaf spot on living leaf of *Brassica rapa* subsp. *chinensis*, collection date and collector unknown (IMI 349061 **epitype of *C. higginsianum***, **here designated**, MBT178519, CBS H-21664 isoepitype, culture ex-epitype IMI 349061 = CPC 18941, CPC 19379); Trinidad, from leaf spot on living leaf of *Brassica rapa subsp. chinensis*, collection date and collector unknown, culture IMI 349063 = CPC 18942, CPC 19380. **USA**, Georgia, experiment, on leaf spots of *Brassica rapa*, 24 Jul. 1916, B. B. Higgins (no. 340), (BPI 398582 **holotype of *C. higginsianum***).

Notes: Colletotrichum higginsianum is known as the causal organism of anthracnose disease of a wide range of cruciferous plants (*Brassicaceae*) and causes mainly leaf spots but also attacks stems, petioles, seed pods and even roots, and is especially destructive in the south Atlantic and Gulf states of the USA (Higgins 1917, Rimmer 2007), but also occurs in the Caribbean and south-east Asia (Birker *et al.* 2009).

Higgins (1917) noted that this species was associated with a leaf spot disease of turnips (*Brassica rapa*) in various localities in Georgia, USA and tentatively called it *C. brassicae* Schulzer & Sacc. However, Higgins had doubts about this identification and sent specimens to P.A. Saccardo. In a footnote, Higgins explained that Saccardo considered that the fungus was a new species and added Saccardo's species description from the note he received after his paper was ready for publication.

A specimen of *C. higginsianum* was located at BPI that was collected by B.B. Higgins prior to the publication (BPI 398582), and is therefore considered as the holotype. The specimen comprises two leaves with leaf spots that agree with the description and figures in the publication. Conidia of the holotype are nearly straight, sometimes very slightly curved, measuring (14–)16–20(–22) × (3–)3.5–4.5(–5) µm, av. ± SD = 18.1 ± 1.9 × 4.1 ± 0.6 µm, L/W ratio = 4.5. This agrees with the shape and measurements of the isolates studied here.

The only species that was described on *Brassica* prior to Higgins (1917) is *C. brassicae* Schulzer & Sacc. (1884), on *Brassica oleracea* v. *caulocarpa* from Vinkovce, Slovenia that forms curved conidia 19–24 µm long (Schultzer von Mueggenburg & Saccardo 1884). The ITS sequence (GenBank EU400155) of strain DAOM 116226 identified as *C. brassicae* (Chen *et al.* 2007) is identical to that of the ex-type strain of *C. spaethianum* (CBS 167.49) from a study on *Colletotrichum* species with curved conidia (Damm *et al.* 2009). Another strain from a stump of *Brassica* sp. in the Netherlands included in the study of Damm *et al.* (2009) was identified as *C. truncatum*, based on sequence similarities with the ex-epitype strain of that species. It is possible that *C. brassicae* is synonymous with either *C. spaethianum* or *C. truncatum*. We have not studied the type specimen as we do not consider this species to be part of the *C. destructivum* species complex. *Colletotrichum brassicicola* was described recently from *Brassica*; it forms straight conidia and belongs to the *C. boninense* species complex (Damm *et al.* 2012).

A species described from leaf spots on *Matthiola incana* in Romania by Sandu-Ville (1959), *C. mathiolae*, also resembles *C. higginsianum* and could be a synonym of this species or closely related based on similar conidia shape and size. *Colletotrichum mathiolae* also forms conidia that are straight to slightly curved, measuring 12–21 × 3–4 µm. The two isolates from *Matthiola* in Japan are closely related to *C. higginsianum*, but do not have the same GAPDH and HIS3 sequences, which may explain why they do not form a stable clade in our phylogeny. Additional isolates from this host, especially from Romania, are required to determine species boundaries and its affinity to *C. mathiolae*.

Colletotrichum higginsianum was regarded as a synonym of *C. gloeosporioides* by von Arx (1957), but Sutton (1980, 1992) considered it as a distinct species based on its conidial morphology and consistent association with cruciferous hosts. O'Connell *et al.* (2004) recognised the similarity and relatedness with *C. destructivum* and regarded *C. higginsianum* as a synonym of *C. destructivum* based on ITS sequences. *Colletotrichum higginsianum* is confirmed as a distinct species in the present study.

Two isolates included in this study, strain CBS 124249 from *Centella asiatica* in Madagascar (Rakotoniriana *et al.* 2008) and strain IMI 391904 from *Raphanus raphanistrum* in Tunisia (Djebali *et al.* 2009), which were previously identified as *C. higginsianum*, were re-identified as *C. tabacum* and *C. lini*, respectively.

O'Connell *et al.* (2004) observed the two-stage hemibiotrophic infection process of *C. destructivum* (re-identified here as *C. higginsianum*) from *Brassica rapa* subsp. *chinensis* on *Arabidopsis thaliana*. They also established an *Agrobacterium*-mediated DNA-transformation system for this fungus. The *Arabidopsis-Colletotrichum* pathosystem provides a model for molecular analysis of plant-fungal interactions in which both partners can be genetically manipulated. This pathosystem has been intensively studied in recent years (Huser *et al.* 2009, Ushimaru *et al.* 2010, Kleemann *et al.* 2012). The genome and *in planta* transcriptome of *C. higginsianum* strain IMI 349063 were sequenced (O'Connell *et al.* 2012), and this is one of the strains included in the present study.

Colletotrichum higginsianum can be identified by TUB2 and ITS sequences. However, there is only one nucleotide difference to the TUB2 sequences of the two unnamed strains from *Matthiola* (CM90-M1 and CM93-M1) and one nucleotide difference to the ITS sequences of *C. tabacum*, respectively. Three of the strains diverge with a further single nucleotide difference to the other *C. higginsianum* strains.

The ITS of strain IMI 349061 was identical with those of *C. higginsianum* isolates 05131 from *Eruca* in the USA (GenBank KF550281, Patel *et al.* 2014), 12-223 (GenBank JX997428, K.S. Han *et al.*, unpubl. data), C97027 and C00112 (GenBank GU935870, GU935872, Choi *et al.* 2011) from *Brassica* probably in Korea, IMI 349063 and MAFF 305635 (GenBank JQ005760, JQ005761 O'Connell *et al.* 2012, Naumann & Wicklow 2013), MAFF 305635, MAFF 238563, MAFF 305970, IFO6182 (GenBank AB042302, AB042303, AB105955, AB105957, Moriwaki *et al.* 2002) from *Brassica*, *Matthiola* and an unknown host, and except for the last, included in this study, and *C. destructivum* isolates RGT-S12, endophyte of *Rumex* probably in China (GenBank HQ674658, Hu *et al.* 2012) and CD-hz 01–CD-hz 03 from *Vigna* in China (GenBank EU070911–EU070913, Sun & Zhang 2009).

Colletotrichum lentis Damm, **sp. nov.** MycoBank MB809921. Fig. 8.

≠ *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore, Phytopathology 25: 121. 1935.

Basionym: *Vermicularia truncata* Schwein., Trans. Amer. Philos. Soc. 4(2): 230. 1832.

≡ *Glomerella truncata* (Schwein.) C.L. Armstrong & Banniza, Mycol. Res. 110: 953. 2006.

Etymology: The species epithet is derived from the host genus *Lens*.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1.5–11 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae or on or close to chains or clusters of pale to dark brown, verruculose, cylindrical to subglobose, cells. *Setae* pale to medium brown, smooth-walled, 40–85 µm long, 1–3-septate, base ± inflated, sometimes constricted at the basal septum, 5–6 µm diam, tip round. *Conidiophores* hyaline, smooth-walled, septate, branched, to 30 µm long. *Conidiogenous cells* hyaline, smooth-walled, cylindrical to ampulliform, 9–28 × 3.5–5 µm, sometimes intercalary (necks not separated from hyphae by septum) and

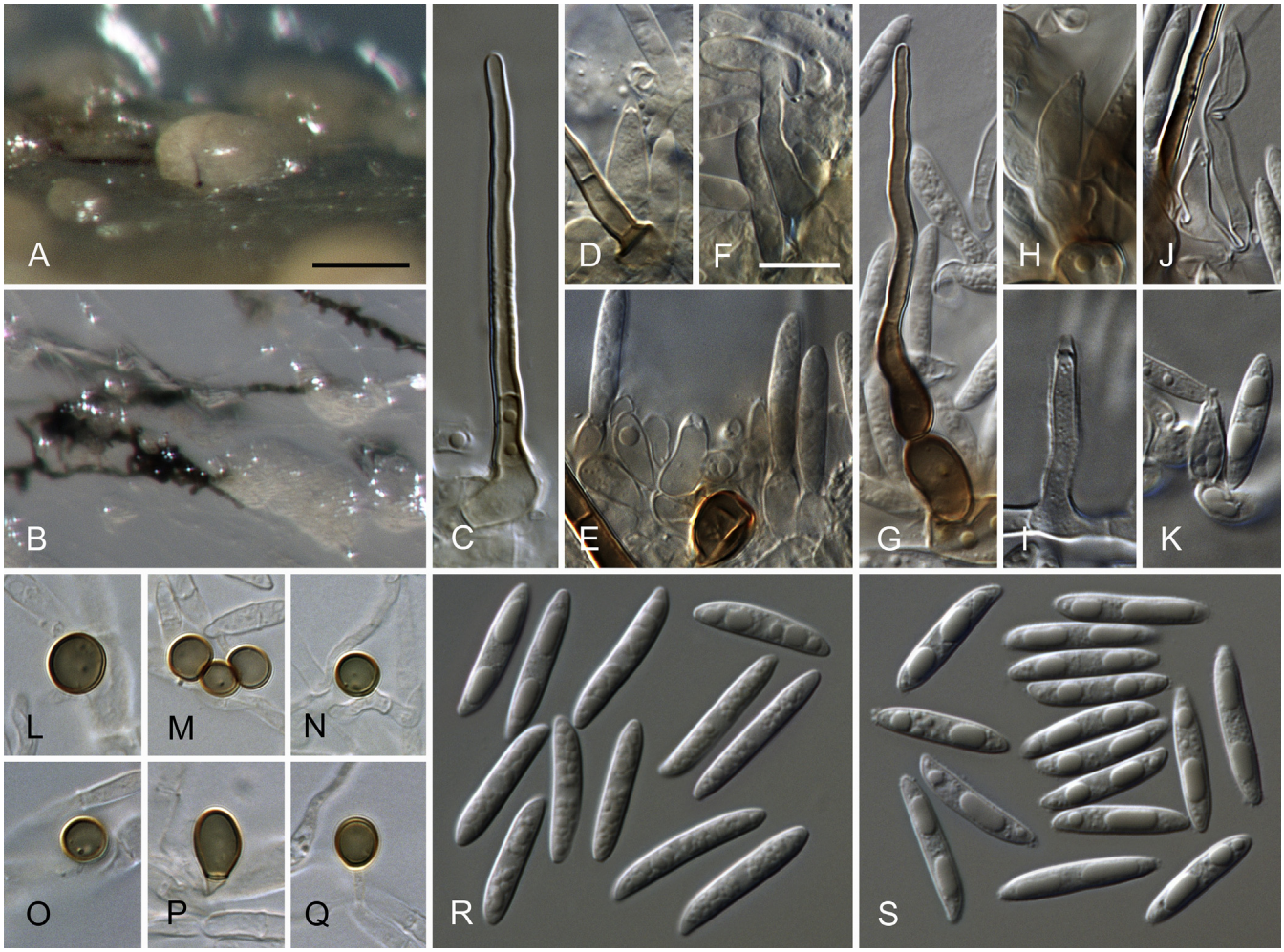

Fig. 8. *Colletotrichum lentis* (from ex-holotype strain CBS 127604). A–B. Conidiomata. C, G. Seta. D–F, H–K. Conidiophores. L–Q. Appressoria. R–S. Conidia. A, C–F, R. from *Anthriscus* stem. B, G–Q, S. from SNA. A–B. DM, C–S. DIC, Scale bars: A = 100 µm, F = 10 µm. Scale bar of A applies to A–B. Scale bar of F applies to C–S.

sometimes polyphialides observed, opening 1–2 µm diam, collarette 0.5–1 µm long, periclinal thickening observed. *Conidia* hyaline, smooth-walled, aseptate straight to slightly curved, fusiform with ± acute ends, (13–)16–20(–26) × 3–4(–5) µm, av. ± SD = 18.1 ± 2.0 × 3.5 ± 0.4 µm, L/W ratio = 5.1, conidia of strain CBS 127605 shorter, measuring (13–)15–17.5(–19.5) × 3–3.5(–4) µm, av. ± SD = 16.3 ± 1.4 × 3.4 ± 0.2 µm, L/W ratio = 4.8. *Appressoria* single or in loose groups, medium brown, smooth-walled, globose, subglobose to elliptical in outline, with an entire margin, (5–)5.5–7.5(–9) × (3.5–)4.5–6(–6.5) µm, av. ± SD = 6.4 ± 0.8 × 5.2 ± 0.6 µm, L/W ratio = 1.2.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on hyaline to pale brown, angular cells, 3.5–9 µm diam. *Setae* pale brown, smooth-walled, 30–120 µm long, 1–3-septate, base ± inflated, 5–6 µm diam, tip round. *Conidiophores* hyaline to pale brown, smooth-walled, septate, branched, to 20 µm long. *Conidiogenous cells* hyaline, smooth-walled, cylindrical to ampulliform, 11–22 × 3.5–5 µm, opening 1.5–2 µm diam, collarette 0.5–1 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, straight to slightly curved, fusiform with ± acute ends, (15.5–)17–20(–21.5) × 3–3.5(–4 µm, av. ± SD = 18.6 ± 1.6 × 3.4 ± 0.3 µm, L/W ratio = 5.5.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline, partly pale rosy buff to pale olivaceous grey, agar medium, filter paper and *Anthriscus* stem partly covered with floccose white aerial mycelium or aerial mycelium lacking, reverse same colours; growth 15–18.5 mm in 7 d (23.5–25 mm in 10 d). Colonies on OA flat with entire margin; surface straw, pale luteous to amber, partly covered with very short aerial mycelium and partly covert with black to salmon acervuli, aerial mycelium lacking, reverse same colours; growth 21–22.5 mm in 7 d (30–34 mm in 10 d). *Conidia* in mass whitish to salmon.

Materials examined: **Canada**, Saskatchewan, North Battlefield, from seed, 2001 crop, sample 90812, of *Lens culinaris* cv. 'CDA Grandora', 2001, R.A.A. Morrall and Discovery Seed Labs (CBS H-21649 **holotype of *C. lentis***, culture ex-holotype CBS 127604 = DAOM 235316 = CT21, Race Ct1); Saskatchewan, Moose Jaw, from seed, 2001 crop, sample 91639, of *Lens culinaris* cv. 'CDA Grandora', 2001, R.A.A. Morrall and Discovery Seed Labs, CBS H-21650, culture CBS 127605 = DAOM 235317 = CT26, Race Ct0. **Romania**, Iaşi, on pods and leaves of *Lens culinaris*, 30 Jun. 1950, C. Sandu-Ville (GLM-F102752 **holotype of *C. savulescui*** Sandu ex Herbarul Micologic "C. Sandu-Ville").

Notes: In 1986 and 1987, an anthracnose disease of lentil (*Lens culinaris*) was observed in Manitoba, Canada, and identified as *C. truncatum* by Morrall (1988). Armstrong-Cho & Banniza (2006) induced the formation of perithecia by crossing single conidial isolates of the lentil pathogen in the laboratory. Consequently, they considered these crosses as the sexual morph of *C. truncatum* and with the dual nomenclature still in place, named this sexual morph *Glomerella truncata*, although morphological as well as molecular studies (Ford *et al.* 2004)

comparing lentil isolates with "*C. truncatum*" isolates from soybean, clover, peanut and cocklebur indicated different species were involved. Latunde-Dada & Lucas (2007) and Gossen *et al.* (2009) found isolates from anthracnose of lentil in Canada to be closely related to *C. destructivum*. Damm *et al.* (2009) epitypified *C. truncatum* and revealed the lentil pathogen from Canada to be a different species. In contrast to that species, *C. truncatum* forms strongly curved conidia and does not belong to the *C. destructivum* complex (Damm *et al.* 2009). The phylogenetic relationship between the two species was demonstrated by O'Connell *et al.* (2012) and Cannon *et al.* (2012).

With the adoption of the new International Code of Nomenclature for algae, fungi and plants concerning species names for morphs with the same epithet introduced prior to 1 January 2013, the name *Ga. truncata* will be considered as a new combination of the previously described *C. truncatum* and not as a new species, although it is based on a different type and the two types are not conspecific (McNeill *et al.* 2012, Hawksworth *et al.* 2013). Consequently, the lentil pathogen from Canada is described as a new species in this study, *C. lentis*. As suggested by Hawksworth *et al.* (2013), the name *Ga. truncata* was treated as a formal error for a new combination and corrected accordingly to *Glomerella truncata* (Schwein.) C.L. Armstrong & Banniza.

The two strains studied here, CBS 127604 and CBS 127605, were crossed to produce the original holotype specimen of "*Ga. truncata*", DAOM 235318, on inoculated sterilised stems of *Lens culinaris*, but the latter will have no nomenclatural status under the new changes to the Code (Hawksworth *et al.* 2013). As there is no strain derived from this specimen and an epitypification would be needed in order to have an ex-type strain, it was not used as holotype of *C. lentis*. Instead, CBS 127604 was chosen for the holotype of *C. lentis*.

A second species from *Lens culinaris*, *C. savulescui*, was described in Romania by Sandu-Ville (1959). As specimen GLM-F102752 is apparently the only specimen of this species from the Herbarium Mycologicum Moldavicum "Constantin Sandu-Ville" (www.uaiasi.ro/agricultura/index.php?lang=en&pagina=pagini/herbarium.html) and no specimen was located elsewhere, it was considered as the holotype of *C. savulescui*, although its collection date was prior to that listed in the publication of Sandu-Ville (1959). Conidia of *C. savulescui* are hyaline, cylindrical with both ends rounded, straight or slightly curved, measuring 7.5–18 × 3–4.5 µm, mostly 12–18 × 4 µm. The size of the conidia found on the holotype is similar to that of *C. lentis*; the conidia are also described as straight to slightly curved, which indicates this species might belong to the *C. destructivum* species complex. However, *C. lentis* has conidia that are fusiform with ± acute ends. The name of this species, as far as we know, has not been used since its original description.

Additionally, isolates from lentil were also included in the study of Liu *et al.* (2013a), identified as *C. nigrum*. *Colletotrichum nigrum* is not closely related to the species treated here and forms entirely straight conidia.

Armstrong-Cho & Banniza (2006) observed self-sterility of all isolates tested, while many pairings produced perithecia and concluded the species to have a homothallic mating system. The study by Menat *et al.* (2012) confirmed a bipolar mating system, however an atypical one, with the HMG box that is part of the MAT1-2 idiomorph being present in both incompatibility groups. The sexual morph was described by Armstrong-Cho & Banniza (2006) as follows "Perithecia were brown-black, superficial, solitary or in small groups, obpyriform to ovate or ampulliform, 200–520 × 110–320 µm (mean: 350 × 200 µm). Asci were cylindrical, narrowing slightly at the apex, unitunicate, evanescent, 53–142 × 5–14 µm (mean: 90 × 8 µm), and contained eight ascospores. Ascospores were hyaline, aseptate, oblong, 12–20 × 5–8 mm (mean: 15.7–6.7 µm)."

Buchwaldt *et al.* (2004) identified two physiological races of "*C. truncatum*" from lentil on the basis of their pathogenicity on a number of lentil cultivars and germplasm lines in western Canada, designating them Ct0 and Ct1.

The intracellular hemibiotrophic infection of the lentil pathogen was studied by Latunde-Dada & Lucas (2007) and Armstrong-Cho *et al.* (2012). This pathosystem was used to identify secreted effector proteins expressed at the switch from biotrophy to necrotrophy (Bhadauria *et al.* 2011) and functional analysis of a nudix hydrolase effector eliciting plant cell death (Bhadauria *et al.* 2012).

Strains that are morphologically similar and molecularly closely related (based on ITS) to *C. lentis* were isolated from the noxious weed scentless chamomile (*Tripleurospermum inodorum*) in Canada (Forseille 2007). The potential of this fungus for biocontrol of scentless chamomile was tested (Peng *et al.* 2005, Forseille *et al.* 2009). In the field, chamomile isolates caused symptoms on its original host but not on lentil or pea. Forseille *et al.* (2009) also observed the hemibiotrophic infection process of this fungus, which might represent a further species of the *C. destructivum* complex.

Colletotrichum lentis is characterised by its slightly curved, fusoid conidia that are gradually tapering to the ± acute ends and by the ± globose appressoria with an entire margin. It can be identified by all loci included in this study.

The ITS sequence of strain CBS 127604 matched in a blastn search with the same sequence (GenBank JQ005766, O'Connell *et al.* 2012) and that of "*C. truncatum*" isolate 9969473 (GenBank AF451902, Ford *et al.* 2004) and with 99 % identity (1–3 nucleotides difference) with "*C. truncatum*" isolates 95S25, 9971646, 95A8, 9970034 from lentil in Canada (GenBank AF451901, AF451904, AF451900, AF451903, Ford *et al.* 2004) and "*Ga. glycines*" isolate IFO7384 from an unknown host (GenBank AB057435, Moriwaki *et al.* 2002). The only matching TUB2 sequence found in GenBank is that of the same strain (GenBank JQ005850, O'Connell *et al.* 2012); all other TUB2 sequences are ≤95 % identical.

Colletotrichum lini (Westerd.) Tochinai, J. Coll. Agric. Hokkaido Imp. Univ. 14: 176. 1926. Fig. 9.

Basionym: *Gloeosporium lini* Westerd., Jaarversl. Phytopathol. Lab. "Willie Commelin Scholten" 6. 1916 [1915].

= *Colletotrichum linicola* Pethybr. & Laff. [as '*linicolum*'], Sci. Proc. Roy. Dublin Soc. 15: 368. 1918.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1.5–6 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores formed directly on hyphae. *Setae* not observed. *Setae* of strain IMI 391904 medium brown, smooth-walled to verruculose, 52–94 µm long, 1–3-septate, base cylindrical to conical, 3.5–6.5 µm diam, tip rounded. *Conidiophores* hyaline, smooth-walled, septate, branched, to 40 µm long. *Conidiogenous cells* hyaline, smooth-walled, cylindrical, 9–32 × 2.5–4.5 µm, opening 1–1.5 µm diam, collarette 0.5 µm long, periclinal thickening rarely observed. *Conidia* hyaline, smooth-walled, aseptate, fusiform, slightly curved to straight, tapering to the

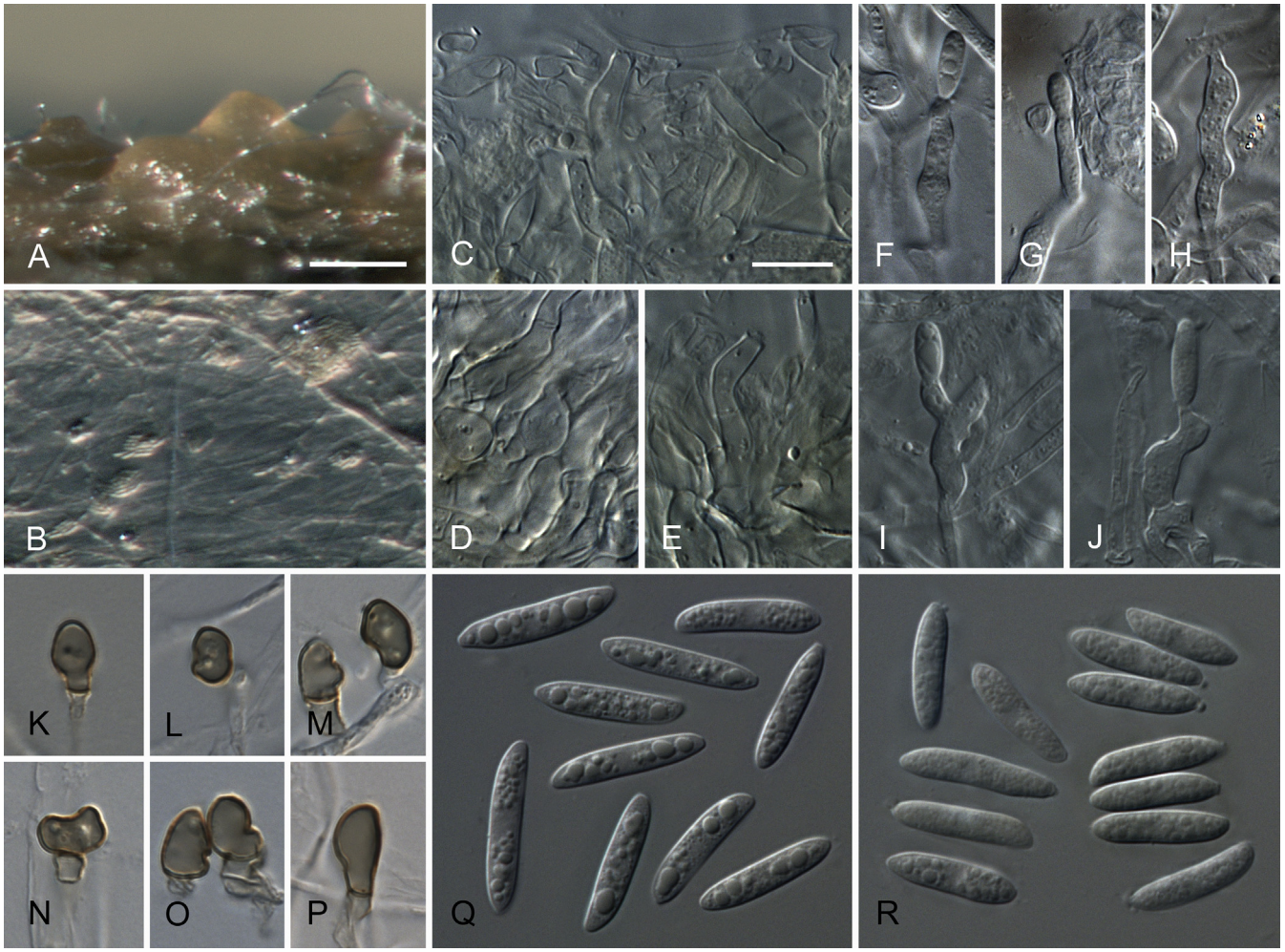

Fig. 9. *Colletotrichum lini* (from ex-epitype strain CBS 172.51). A–B. Conidiomata. C–J. Conidiophores. K–P. Appressoria. Q–R. Conidia. A, C–E, Q. from *Anthriscus* stem. B, F–P, R. from SNA. A–B. DM, C–R. DIC, Scale bars: A = 100 µm, C = 10 µm. Scale bar of A applies to A–B. Scale bar of C applies to C–R.

slightly rounded to acute ends, (13–)15–18(–22.5) × (3–)3.5–4(–4.5) µm, av. ± SD = 16.6 ± 1.6 × 3.8 ± 0.3 µm, L/W ratio = 4.4, conidia of strain CBS 112.21 are smaller, measuring (12–)13.5–16.5(–18.5) × (3–)3.5–4.5(–5) µm, av. ± SD = 15.0 ± 1.4 × 4.0 ± 0.4 µm, L/W ratio = 3.7, conidia of strain CBS 117156 are longer, measuring (18–)18.5–20(–21) × 3.5–4(–4.5) µm, av. ± SD = 19.3 ± 0.8 × 3.9 ± 0.2 µm, L/W ratio = 5.0, the ex-epitype strain CBS 172.51 and of strain CBS 112.21 formed inside SNA agar medium are larger conidia than on the surface of the medium, those of strain CBS 172.51 measure (23.5–)24–33(–52.5) × 4–4.5(–5) µm, av. ± SD = 28.6 ± 4.3 × 4.3 ± 0.3 µm, L/W ratio = 6.7. *Appressoria* single or in loose groups, pale brown, smooth-walled, ellipsoidal to subglobose outline, with an entire or undulate margin, (5–)6.5–10(–12.5) × (4–)4.5–6(–7) µm, av. ± SD = 8.3 ± 1.9 × 5.3 ± 0.9 µm, L/W ratio = 1.6, strain IMI 391904 additionally formed appressoria-like structures within the mycelium, measuring (3.5–)5–7.5(–8) × (2.5–)3.5–5.5(–6) µm, av. ± SD = 6.3 ± 1.2 × 4.5 ± 0.8 µm, L/W ratio = 1.4.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on pale brown, angular cells, 3–8.5 µm diam. *Setae* not observed. *Setae* of strain IMI 391904 medium brown, smooth-walled to finely verruculose, 55–210 µm long, 1–5(–6)-septate, base cylindrical to conical, 3.5–7 µm diam, tip slightly rounded. *Conidiophores* hyaline, smooth-walled, septate, branched, to 40 µm long. *Conidiogenous cells* hyaline, smooth-walled, cylindrical to elongate ampulliform, 8–22 × 2.5–4 µm, opening 1–2 µm diam, collarette 0.5–1 µm long, periclinal thickening observed. *Conidia* hyaline, smooth-walled, aseptate, fusiform, slightly curved to straight, tapering to the slightly rounded to acute ends, (14.5–)16.5–19.5(–21.5) × 3.5–4 µm, av. ± SD = 18.0 ± 1.5 × 3.8 ± 0.2 µm, L/W ratio = 4.7, conidia of strain CBS 112.21 are smaller, measuring (13.5–)15–17.5(–19.5) × 4–4.5 µm, av. ± SD = 16.3 ± 1.4 × 4.3 ± 0.2 µm, L/W ratio = 3.8, conidia of strain CBS 117156 are longer (17.5–)19.5–22.5(–23.5) × (3–)3.5–4(–4.5) µm, av. ± SD = 21.1 ± 1.4 × 3.8 ± 0.2 µm, L/W ratio = 5.5.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline to pale luteous, filter paper partly pale luteous, agar medium and *Anthriscus* stem partly covered with floccose white aerial mycelium, reverse same colours; growth 27.5–30 mm in 7 d (>40 mm in 10 d). Colonies on OA flat with entire margin; buff to rosy-buff, aerial mycelium lacking, reverse buff, growth 26–29 mm in 7 d (37.5–>40 mm in 10 d). *Conidia* in mass not observed.

Materials examined: **Germany**, Dierhagen-Neuhaus, walkway, from stems and leaves with black spots of *Trifolium repens*, 4 Aug. 2010, U. Damm, CBS H-21660, culture CBS 130828. **Ireland**, from *Linum usitatissimum*, collection date unknown (isolated by P. Mercer and deposited in CBS collection Feb. 1997 by J.A. Bailey), P. Mercer, culture CBS 505.97 = LARS 77. **Netherlands**, from leaves and stems of *Linum* sp., collection date and collector unknown (IMI 194722 ex coll. Prof. J. van Westerdijk, **lectotype of *Gm. lini*, here designated**, MBT178721); from seed plants of *Linum* sp., collection date and collector unknown (IMI 194721 ex coll. Prof. J. van Westerdijk); from seedling disease of *Linum usitatissimum*, collection date and collector unknown (deposited in CBS

collection Sep. 1951 by Plantenziektenkundige Dienst Wageningen, Nederland, identified by A.C. Stolk) (CBS H-21657 **epitype of *Gm. lini*, here designated**, MBT178521, culture ex-epitype CBS 172.51); Province Gelderland, Malden, closed railway nearby gliding-club, from leaf spots of *Teucrium scorodonia*, 23 Aug. 2004, G. Verkley and M. Starink, V3037, culture CBS 117156. **New Zealand**, from *Trifolium* sp., collection date and collector unknown, (history: I.D. Blair, 1957 CABI), culture IMI 69991 = CPC 20242. **Tunisia**, site Barragage Jjoumine, from symptoms on a living leaf of *Raphanus raphanistrum*, collection date and collector unknown (deposited in IMI collection by Dr. M. Jourdan), CBS H-21658, culture IMI 391904 = CPC 19382 = IS320. **UK**, from seedling disease of *Linum usitatissimum*, collection date and collector unknown (isolated by G.H. Pethybridge, deposited in CBS collection Aug. 1921 by G.H. Pethybridge), CBS H-21656, culture CBS 112.21 = LCP 46.621. **USA**, Utah, Salt Lake City, cemetery, from small black spots on petioles of *Trifolium hybridum*, 24 Aug. 2013, U. Damm, CBS H-21659, culture CBS 136850; Utah, Bluffdale near Salt Lake City, stems of *Medicago sativa*, 25 Aug. 2013, U. Damm, culture CBS 136856.

Notes: Anthracnose has a serious impact on yield and fibre quality of flax (*Linum usitatissimum*) and is well-known in Europe, Asia and America. Flax anthracnose increased in Germany when flax production was expanding in the 1930s (Rost 1938). The anthracnose pathogen is seed- and soilborne, causes damping off of flax seedlings (Rost 1938), and is one of the causal organisms of so-called flax-sick soils (Bolley & Manns 1932).

Van Westerdijk (1916) described the flax anthracnose pathogen in the Netherlands as *Gloeosporium lini*, citing the genus as *Gloeosporium* (*Colletotrichum*). This name was combined into *Colletotrichum* by Tochinai (1926), following the study of several Japanese collections. Neither the location of the fungus nor a type was listed by van Westerdijk. Unfortunately, no strain was preserved in the CBS culture collection. However, two specimens from Van Westerdijk's *Gm. lini* collections were sent to the IMI fungarium by von Arx, and the one containing a larger amount of diseased plant material (IMI 194722) is designated as lectotype. The specimen includes fusiform, slightly curved to straight conidia with slightly rounded to acute ends that measure (14–)16–21(–24) × (3–)3.5–5 µm, av. ± SD = 18.4 ± 2.3 × 4.2 ± 0.7 µm, L/W ratio = 4.4. Sutton (1980) listed the species from flax as *C. lini* (Westerd.) Tochinai, but later (Sutton 1992) followed Dickson's (1956) opinion that the basionym, *Gm. lini* Westerd. was probably synonymous with *Polyspora lini* Laff. (current name in Species Fungorum: *Kabatiella lini*) and not a *Colletotrichum*. However, the conidia on the lectotype specimen of *Gm. lini* agreed both in shape and size with the *Colletotrichum* species from *Linum* we treat in this study.

A *Colletotrichum* species on *Linum* in North Dakota, USA, was studied between 1901 and 1903 by T.F. Manns and also called *C. lini*; however, his thesis was never published (Manns & Bolley 1932). The name was taken up by Bolley (1910); however, it is illegitimate as it is a "*nomen nudum*". Bolley & Manns later (1932) treated the fungus as *C. lini* Manns et Bolley. Conidia of this species measure 15–20 × 2–4.5 µm, setae are 70–130 µm long and 2–4-septate, and the olive brown "chlamydospores" measure 10–15 × 10–12 µm (Bolley & Manns 1932). This agrees with the observations of the *Colletotrichum* from *Linum* in this study and is probably a synonym. We have not seen the type of this species and no isolates from *Linum* in the USA were available to us.

Pethybridge & Lafferty (1918) described *C. linicola* as the causal agent of damping off of flax seedlings in Ireland with conidia measuring 17 × 4 µm and 3-septate setae measuring 150 × 4 µm. This species is most probably a synonym of *C. lini* (Westerd.) Tochinai. Both an authentic strain from the UK isolated by G.H. Pethybridge (CBS 112.21) and a strain from Ireland (CBS 505.97) are included in our study.

Rost (1938) lists *C. atramentarium* that formed straight conidia on flax in Germany and which is probably a synonym of *C. coccodes* (Liu *et al.* 2011). Wollenweber & Hochapfel (1949) also identified a collection from stems of *Linum* from Silesia as *C. atramentarium*.

Hahn (1952) examined the infection process of *C. lini* on resistant and susceptible flax lines and provided the first description of bulbous primary hyphae colonising single epidermal cells. These were subsequently found to be the characteristic biotrophic infection structures formed by all members of the *C. destructivum* species complex examined to date.

Conidia of *C. lini* strains from *Linum* are similar to those of *C. lentis*. They are both slightly curved and fusiform, but conidia of *C. lini* are more abruptly tapering to the slightly acute ends; this shape was noticed in the type material (not shown), and very long conidia were found within the agar medium. In accordance with the original description and the observations on the type, no setae were observed on the strains from flax, but none of the isolates was recently collected.

In contrast to the *C. lini* strains from *Linum*, the strains from *Trifolium hybridum*, *T. repens*, *Medicago sativa* and *Taraxacum* sp. formed setae and rather cylindrical conidia with rounded ends. These strains formed a subclade within *C. lini*. However, we refrained from describing these strains as a new species, because there was only one nucleotide difference in the TUB2 sequence to separate them from the remaining *C. linum* strains; the overall sequence variability within *C. lini* was higher. Moreover, their morphology was similar to strains from *Nigella*, *Raphanus* and *Teucrium*, which belong to the same subclade as the strains from *Linum*. Both subclades contain strains from multiple hosts.

Colletotrichum lini is distinguishable by CHS-1, HIS3, ACT and TUB2. The ITS and GAPDH sequences are the same as those of *C. americae-borealis*. The sequences of all genes in strains from *Linum*, *Nigella* and *Teucrium* are identical. The strain from *Raphanus* in Tunisia (IMI 391904) with the longest branch differs only in its GAPDH sequence.

As the sequences are the same, blastn searches with the ITS sequence of *C. lini* strain CBS 172.51 resulted in the same matching sequences as those with the ITS of *C. americae-borealis*, including isolates from alfalfa, clover, *Oxytropis*, endophytes from *Holcus* and *Arabidopsis* as well as strain IMI 391904 that is included in our study and a strain from *Convolvulus* in Turkey. Strain IMI 391904 originated from a study on pathogenic fungi on wild radish (*Raphanus raphanistrum*) in northern Tunisia in order to screen for potential biocontrol agents against this weed (Djebali *et al.* 2009). It was previously identified as *C. higginsianum* and re-identified as *C. lini* in this study. The identification of the strain from field bindweed (*Convolvulus arvensis*) in Turkey as *C. linicola* is based on the ITS sequence only (Tunali *et al.* 2008); it was tested to be effective as a potential biocontrol agent against that plant (Tunali *et al.* 2009). However, the identity of this strain needs to be confirmed with sequences of additional loci.

The ITS sequence of *C. lini* strain Coll-44 from a recent disease report of anthracnose on *Medicago* in Serbia (GenBank JX908364, Vasić *et al.* 2014) is identical to strains from *C. americae-borealis* and *C. lini*. As the TUB2 sequence (GenBank KJ556347, kindly provided by Tanja Vasić) was identical to that of *C. lini* strain CBS 136850 (from *Trifolium hybridum*, USA), strain Coll-44 is confirmed as *C. lini*. Sequences of an isolate

from our study (CBS 157.83) and several ITS sequences detected in GenBank (GenBank JX908362, JX908363, JX908361, Vasić, unpubl. data) are identical to those of *C. destructivum s. str.*, indicating the occurrence of at least two species on *Medicago* in Serbia.

The performance of *C. lini* strain CBS 112.21 in comparison with *Botryodiplodia malorum* in steroid hydroxylations, to improve the biotransformation of steroids for the pharmaceutical industry, was studied by Romano *et al.* (2006).

Colletotrichum ocimi Damm, **sp. nov.** MycoBank MB809401. Fig. 10.

Etymology: The species epithet is derived from the host genus name *Ocimum*.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–7 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* conidiophores and setae formed on pale brown, roundish cells, 5–22 µm diam. *Setae* medium brown, smooth-walled to verruculose, 43–103 µm long, 1–2-septate, base cylindrical to ± inflated, 4.5–9.5 µm diam, tip ± rounded to ± acute. *Conidiophores* hyaline, smooth-walled, septate, branched, to 60 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled to verruculose, cylindrical to clavate, sometimes intercalary (necks not separated from hyphae by septum), often with slime sheaths, 10.5–24 × 3.5–5.5 µm, opening 1–1.5 µm diam, collarette 0.5–1.5 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, straight, cylindrical, with both ends rounded or one end round and the other truncate, (13.5–)14.5–15.5(–16.5) × (3.5–)4–4.5 µm, av. ± SD = 15.0 ± 0.7 × 4.1 ± 0.2 µm, L/W ratio = 3.7. *Appressoria* very few, single, scattered, pale brown, smooth-walled, ellipsoidal, clavate, subglobose or irregular outline, with a lobate or entire margin, (6.5–)7–13(–15.5) × (4–)4.5–7.5(–9) µm, av. ± SD = 9.9 ± 2.9 × 6.0 ± 1.3 µm, L/W ratio = 1.6.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on pale brown, verruculose, roundish cells, 4–17 µm diam. *Setae* medium brown, verruculose, 30–145 µm long, 1–4-septate, base cylindrical, conical to ± inflated, 4–7.5 µm diam, tip ± rounded to ± acute. *Conidiophores* hyaline to pale brown, smooth-walled, septate, branched, to 25 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical to ampulliform, 8–21.5 × 3.5–5 µm, opening 1–1.5 µm diam, collarette 0.5 µm long, periclinal thickening visible. *Conidia* hyaline, smooth-walled, aseptate, straight, cylindrical, with both ends rounded or one end round and the other truncate, (11–)14–16(–16.5) × (3.5–)4(–4.5) µm, av. ± SD = 14.8 ± 1.0 × 4.0 ± 0.2 µm, L/W ratio = 3.7.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline to cinnamon, agar medium, filter paper and *Anthriscus* stem partly covered with grey acervuli, aerial mycelium lacking, reverse same colours; growth 18–19.5 mm in 7 d (28–29.5 mm in 10 d). Colonies on OA flat with entire margin; buff to honey, almost entirely covert with dark grey to black acervuli and salmon conidial masses, aerial mycelium lacking, reverse rosy buff, vinaceous buff to pale olivaceous-grey, growth 20.5–22 mm in 7 d (30–31.5 mm in 10 d). *Conidia in mass* salmon.

Material examined: **Italy**, Riviera Ligure, from a black spot on leaf of *Ocimum basilicum*, collection date and collector unknown (deposited in CBS collection May 1994 by A. Garibaldi, Inst. degli studi di Torino, Depart. di Valorizzazione e Protezione delle Risore agroforestiali) (CBS H-21646 **holotype**, culture ex-holotype CBS 298.94).

Notes: Basil (*Ocimum basilicum*) is an aromatic culinary herb, for which flawless leaves are of special importance. Gullino *et al.* (1995) reported an outbreak of a new foliar disease of basil cultivated in greenhouses in northern Italy and consistently isolated a *Colletotrichum* species. The fungus caused black spots on stems and leaves of basil; lesions on stems often resulted in girdling and plant death. One strain (CBS 298.94) was sent to CBS and identified as *Glomerella cingulata var. cingulata* (until recently regarded as the sexual stage of *C. gloeosporioides*) by H.A. van der Aa (HA 11925) as indicated in the database of the CBS culture collection.

This species forms cylindrical, straight conidia with round ends, reminiscent of species in the *C. gloeosporioides* complex (Weir *et al.* 2012). However, we found that *C. ocimi* belongs to the *C. destructivum* species complex. Gullino *et al.* (1995) did not observe a sexual stage of the basil fungus. Apart from the conidia, *C. ocimi* differs from the other species in the *C. destructivum* complex by its conidiogenous cells that are often covered by mucoid sheaths.

No species were previously described on *Ocimum*. Additionally to Gullino *et al.* (1995), Farr & Rossman (2014) list a few further reports of *Colletotrichum* species on basil: *C. capsici* in India, *C. gloeosporioides* in Cambodia and *Colletotrichum* sp. in Florida, USA. It is possible that the latter two reports refer to *C. ocimi* as well. However, the only sequence of a *Colletotrichum* strain from basil in GenBank, which is an ITS sequence of strain EGJMP 40 probably from India (GenBank KF234012) identified as *C. aotearoa* (E.G. Jagan *et al.*, unpubl. data), indeed refers to a species belonging to the *C. gloeosporioides* species complex.

This species can be identified by its unique ITS, CHS-1, HIS3, ACT, and TUB2 sequences. The closest match in a blastn search with the ITS sequence of strain CBS 298.94 is GenBank EU400148 from *C. lini* strain DAOM 183091 (Chen *et al.* 2007). No TUB2 sequences were detected in GenBank with >97 % identity. The GAPDH sequence of *C. ocimi* is the same as that of *C. destructivum* (*s. str.*).

Colletotrichum panacicola Uyeda & S. Takim., Bull. Korean Agric. Soc. 14: 24. 1919. MycoBank MB809665.

≡ *Colletotrichum panacicola* Uyeda & S. Takim., Bull. Agric. Experiment Stat. Chosen (Korea) 5: 16. 1922. Nom. illegit., Art. 53.1.

Notes: Colletotrichum panacicola, originally described from *Panax ginseng* in Korea, has also been reported from China, eastern Russia and Japan, while anthracnose of American ginseng (*P. quinquefolius*) is caused by *C. dematium* (*s. lat.*) and *C. coccodes* (McPartland & Hosoya 1997). McPartland & Hosoya (1997) corrected the author citation of the species that had been described already by Takimoto (1919), but that would have to be cited as *C. panacicola* Uyeda & S. Takim. The confusion was caused by Nakata & Takimoto (1922) who described the same species again as a new species. Petrak (1953) cited the species wrongly as ***C. panacicola*** Nakata & S. Takim., which was subsequently taken up by Index Fungorum.

The species was characterised with aseptate, cylindrical, straight or slightly curved conidia with rounded ends, measuring 17.0–22.1 × 3.4–5.1 µm, pyriform olive coloured appressoria,

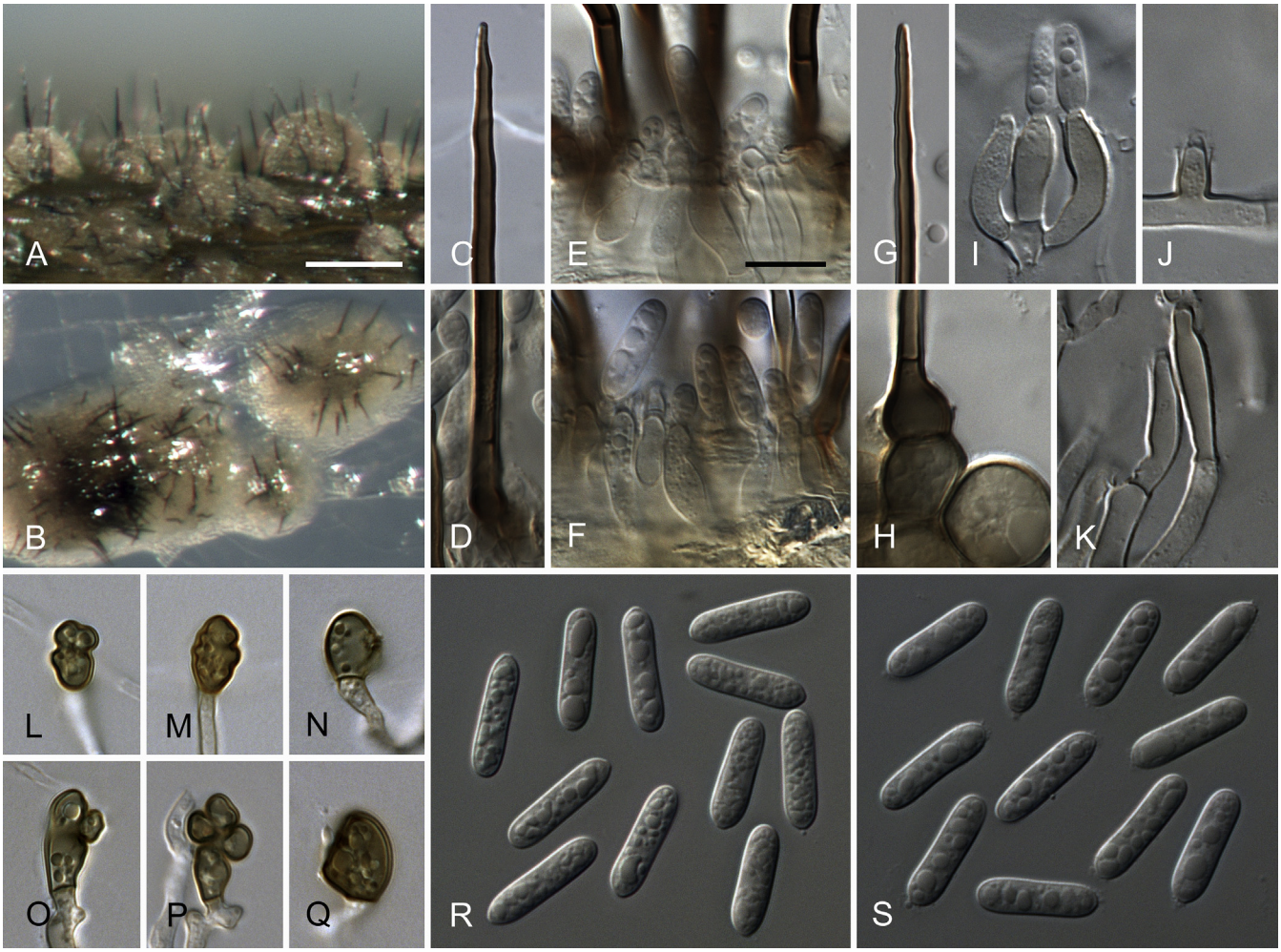

Fig. 10. *Colletotrichum ocimi* (from ex-holotype strain CBS 298.94). A–B. Conidiomata. C, G. Tip of a seta. D, H. Base of a seta. E–F, I–K. Conidiophores. L–Q. Appressoria. R–S. Conidia. A, C–F, R. from *Anthriscus* stem. B, G–Q, S. from SNA. A–B. DM, C–S. DIC, Scale bars: A = 100 µm, E = 10 µm. Scale bar of A applies to A–B. Scale bar of E applies to C–S.

measuring 14–8 µm and dark olive 1–3-septate setae with acute paler apices that measure 31–144 × 2.4–8.4 µm (Takimoto 1919, Nakata & Takimoto 1922, both cited by McPartland & Hosoya 1997). McPartland & Hosoya (1997) were unable to locate either type or authentic specimens. As the illustration in Nakata & Takimoto (1922) is not sufficiently diagnostic to act as a lectotype, the species needs to be neotypified.

Fresh cultures are available as Choi *et al.* (2011) recently studied isolates of this species and observed similarity with *C. higginsianum*, *C. destructivum* and *C. coccodes*. ITS sequences did not distinguish the species from *C. higginsianum* and *C. destructivum*. The inclusion of more genes (ACT, translation elongation factor 1-α, glutamine synthase) clearly showed this species to be different from the other two (Choi *et al.* 2011). Choi *et al.* (2011) who also showed this species to only infect Korean ginseng, suggesting it was a distinct taxon.

As there were no isolates available to us, we could not directly compare the morphology of *C. panacicola*. However, we included DNA sequences of three isolates from the study of Choi *et al.* (2011) that were retrieved from GenBank in our molecular analyses (only with ITS, GAPDH and ACT), which confirmed *C. panacicola* to belong to the *C. destructivum* complex and to be a distinct species, although closely related to the newly described *C. utrechtense*, which has the same ACT sequence. *Colletotrichum panacicola* can be identified by ITS and GAPDH sequences; 100 % sequence identities on GenBank were only found with the *C. panacicola* sequences from the study of Choi *et al.* (2011). Unfortunately, the TUB2 region sequenced by Choi *et al.* (2011) was different from the region we studied and could not be compared to our dataset; the CHS-1 and HIS sequences of this species were not available for comparison.

Colletotrichum pisicola Damm, **sp. nov.** MycoBank MB809403. Fig. 11.

Etymology: The species epithet is derived from the host plant genus, *Pisum*.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–7.5 µm diam, hyaline, smooth-walled, septate, branched, at some parts pale to medium brown. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae or aggregated on clusters of pale to medium brown, roundish cells, 3.5–11 µm diam. *Setae* few observed, pale brown, smooth-walled to verrucose, 30–40 µm long, 1–2-septate, base cylindrical to conical, 4–5 µm diam, tip round or with a conidiogenous locus. *Conidiophores* hyaline, smooth-walled, septate, branched, to 45 µm long. *Conidiogenous cells* hyaline, smooth-walled, cylindrical to ampulliform, sometimes intercalary (necks not separated from hyphae by septum), 11–30 × 2.5–5 µm, opening 1–1,5 µm diam, collarette 1–2,5 µm long, periclinal thickening visible, sometimes distinct. *Conidia*

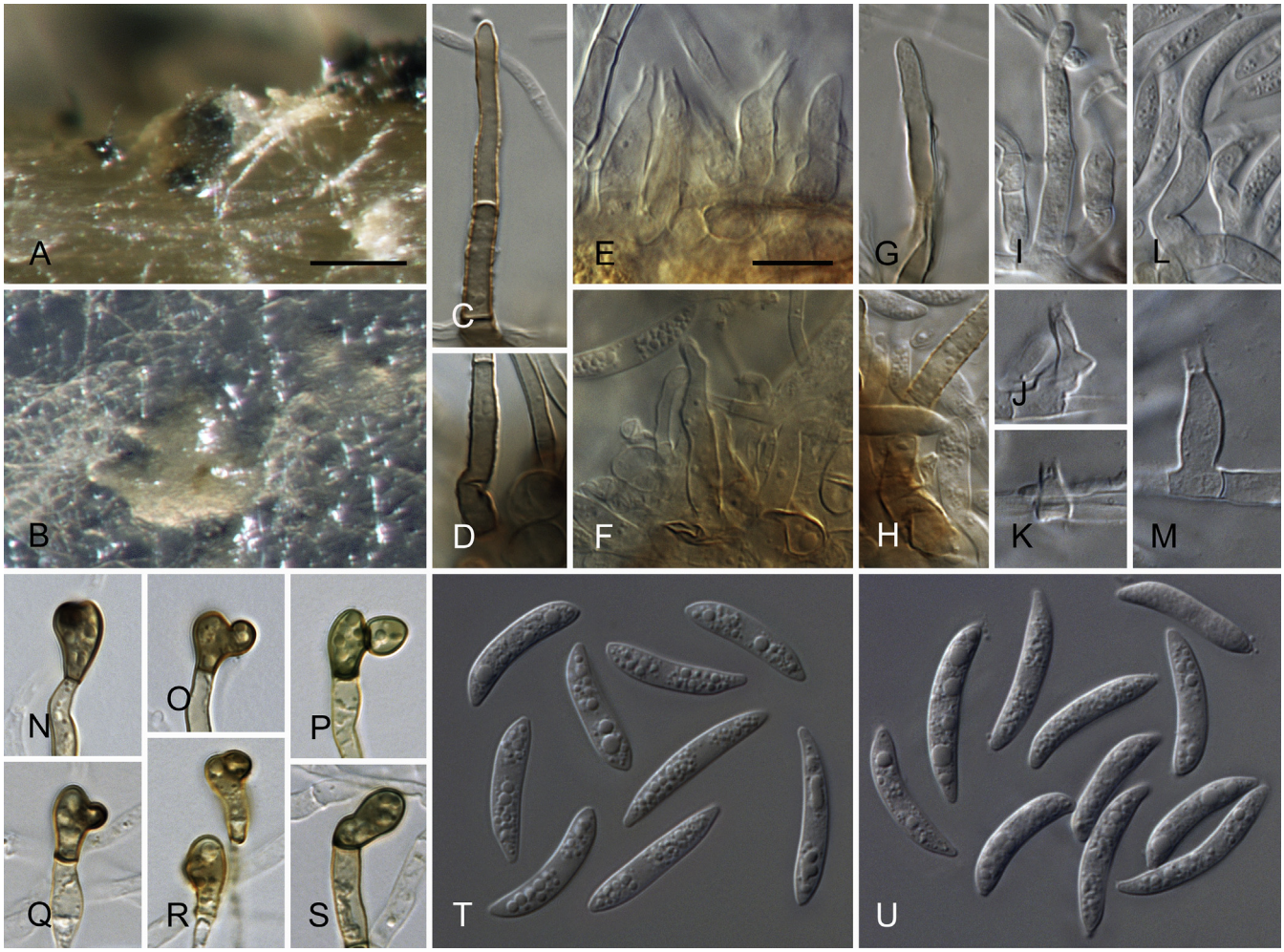

Fig. 11. *Colletotrichum pisicola* (from ex-holotype strain CBS 724.97). A–B. Conidiomata. C, G. Tip of a seta. D, H. Base of a seta. E–F, I–M. Conidiophores. N–S. Appressoria. T–U. Conidia. A, C–F, T. from *Anthriscus* stem. B, G–S, U. from SNA. A–B. DM, C–S. DIC, Scale bars: A = 100 µm, E = 10 µm. Scale bar of A applies to A–B. Scale bar of E applies to C–S.

hyaline, smooth-walled, aseptate, fusiform, distinctly curved gradually tapering to the ± acute ends, (11–) 15–21(–29.5) × (3–)3.5–4 µm, av. ± SD = 18.1 ± 2.9 × 3.5 ± 0.2 µm, L/W ratio = 5.2. *Appressoria* single, pale brown, smooth-walled, elliptical, clavate to irregular outline, with an entire or undulate margin, (5.5–)7–11.5(–13.5) × (4–)4.5–6(–6.5) µm, av. ± SD = 9.3 ± 2.2 × 5.1 ± 0.7 µm, L/W ratio = 1.8.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on pale to medium brown, roundish to angular cells, 3.5–12 µm diam. *Setae* pale brown, smooth-walled to verrucose, 40–55 µm long, 1–2-septate, base conical, 4–6.5 µm diam, tip rounded. *Conidiophores* pale brown, smooth-walled, sometimes septate and branched, to 30 µm long. *Conidiogenous cells* pale brown, smooth-walled, ampulliform to cylindrical, 10.5–24 × 3.5–5.5 µm, opening 1–1.5 µm diam, collarette 0.5–1 µm long, periclinal thickening observed. *Conidia* hyaline, smooth-walled, aseptate, fusoid, distinctly curved, gradually tapering to the ± acute ends, (12–) 15–20.5(–23.5) × (3–)3.5–4 µm, av. ± SD = 17.8 ± 2.8 × 3.7 ± 0.3 µm, L/W ratio = 4.7.

Culture characteristics: Colonies on SNA flat with entire margin, pale straw, covered short filty whitish aerial mycelium, reverse pale luteous; growth 6.5–7.5 mm in 7 d (8.5–10 mm in 10 d). Colonies on OA flat with entire margin; pure yellow to luteous, with a buff margin, covered with very short aerial mycelium, reverse pale luteous to luteous, growth 12–15 mm in 7 d (17.5–20 mm in 10 d). *Conidia* in mass not visible.

Materials examined: **Ecuador**, Quito, from anthracnose symptoms on pods of *Pisum* sp., Jan. 1891, G. Lagerheim (BPI 797146 (ex herbarium N. Patouillard) **lectotype of *C. pisi*, here designated,** MBT178523); Quito, from anthracnose symptoms on pods of *Pisum sativum*, Jan. 1892, G. Lagerheim, BPI 399530, includes slide; Quito, from anthracnose symptoms on pods of *Pisum sativum*, Feb. 1892, G. Lagerheim, BPI 399531, includes slide; Quito, from pods of *Pisum sativum*, Feb. 1892, G. Lagerheim (No. 2944), BPI 399532, includes slide. **Mexico**, intercepted at El Paso, Texas, USA, from anthracnose symptoms on pods of *Pisum sativum*, 17 Dec. 1952, J.A. Baker (No. 53856), BPI 399536; intercepted at Laredo, Texas, USA, from anthracnose symptoms on pods of *Pisum sativum*, 19 Nov. 1954, Ragsdale (No. 55077), BPI 399534. **USA**, Wisconsin, from *Pisum sativum*, collection date unknown (isolated by H.D. van Etten, deposited in LARS collection by D.O. TeBeest, No. 403, deposited in CBS collection Apr. 1997 by J.A. Bailey), H.D. van Etten (CBS H-21644 **holotype of *C. pisicola***, culture ex-holotype CBS 724.97 = LARS 60 = ATCC 64197 = IMI 317934).

Notes: Patouillard & Lagerheim (1891) described *C. pisi* from *Pisum sativum* in Quito, Ecuador with hyaline, fusoid conidia with acute ends, straight to curved, measuring 11–13 × 3–4 µm and setae measuring 60–90 × 6 µm. Three specimens were located in the BPI fungarium that were collected by G. Lagerheim, but only one was collected in 1891. This specimen, BPI 797146 that also originated from the collection of N. Patouillard is designated as the lectotype of *C. pisi* in our study. Conidia found on the

material are fusiform and mostly ± curved and agree with the original description of the species: (10–)11.5–15(–16.5) × (3–)3.5–4(–4.5) µm, av. ± SD = 13.2 ± 1.8 × 3.7 ± 0.4 µm, L/W ratio = 3.5. Conidia found on BPI 399534 were larger, measuring (10–)14–17.5(–20) × (3–)3.5–4.5 µm, av. ± SD = 15.6 ± 1.8 × 3.8 ± 0.5 µm, L/W ratio = 4.1. Whether the specimens collected by G. Lagerheim represent the same species is doubtful.

Among other *C. pisi* specimens in BPI were two (BPI 399534, BPI 399536) that originated from Mexico and were intercepted at the border with the USA. The species on the pea pods and seeds of these specimens were identified as *C. pisi*, although considerably longer conidia were noted; for BPI 399536 the measurements were included in the note on the package as 16–22 × 3–5 µm, which agrees with the observations on strain CBS 724.97 studied here. Hemmi (1921) also observed larger conidia in material on *P. sativum* in Japan compared to those of the original description. He also considered the fungus as *C. pisi*, with straight to slightly curved, fusiform conidia with slightly acute ends. This indicates the presence of at least two *Colletotrichum* species with curved conidia on *Pisum sativum*.

Conidia of strain CBS 724.97 are larger than those of the lectotype of *C. pisi*, measuring (11–)15–21(–29.5) × (3–)3.5–4 µm on SNA compared to (10–)11.5–15(–16.5) × (3–)3.5–4(–4.5) µm of *C. pisi*. The species represented by strain CBS 724.97 is described as new. Regarding conidial size, the specimens from Mexico and Hemmi's Japanese collection resemble *C. pisicola* rather than *C. pisi*.

The existence of at least two *Colletotrichum* species is further supported by the second strain from *Pisum sativum* included in this study, strain CBS 107.40 from Russia. This strain is deposited in CBS as *C. pisi* and belongs to a species closely related to *C. pisicola* (see below *Colletotrichum* sp. CBS 107.40).

Farr & Rossman (2014) report *C. pisi* on *Pisum sativum* in Brazil, China, Canada, USA (Connecticut, Florida, Georgia, Hawaii, Iowa, Idaho, Louisiana, Maine, Minnesota, Texas, Wisconsin), USSR, Guatemala, India and the Malay Peninsula. Hemmi (1921) also reported the species to be common on *P. sativum* in Japan. Further species reported on *P. sativum* (or *P. arvense*) include *C. dematium* from Barbados and Mexico, *C. falcatum* from Hawaii, *C. gloeosporioides* from China, India and USA (North Carolina), *C. lindemuthianum* from Chile, China and Poland, *C. truncatum* from Pakistan and the USA and *Colletotrichum* sp. from Brazil, Malaysia and the USA (Oregon). Hagedorn (1974) reports widespread and serious local damage by pea anthracnose in Wisconsin, USA. We cannot prove which of these reports actually refer to *C. pisicola* as there are no isolates available.

Strain CBS 724.97 was regarded as *C. truncatum e.g.* by Sherriff *et al.* (1994), Shen *et al.* (2001) and Latunde-Dada & Lucas (2007) and is included in the ATCC collection as *C. dematium f. truncatum*. Based on information in the CBS strain database, this strain was also previously identified as *C. destructivum* and as *C. pisi*.

The two *Colletotrichum* strains from *Pisum* represent basal species in the *C. destructivum* complex. This was also observed in preliminary LSU and ITS phylogenies of the genus *Colletotrichum*, in which they formed a sister clade to the other species in this complex (U. Damm, unpubl. data). Consequently, the two species were chosen as outgroup in the phylogeny of the species complex in this study. Their morphological features are not typical for this complex: conidia at least of *C. pisicola* are curved. However, O'Connell *et al.* (1993) investigated the hemibiotrophic infection of *Pisum sativum* by strain LARS 60 (= CBS 724.97, *C. pisicola*) with light and electron microscopy. Both the biotrophic phase and primary hyphae of this fungus were confined to the first infected epidermal cell, but these hyphae were less bulbous and more convoluted than those reported for other members of the *C. destructivum* species complex.

The identification of a strain from roots of *Salix* as *C. pisi* from Corredor *et al.* (2012) based on a blastn search on GenBank with its ITS sequence (Genbank GU934514) is based on another apparently wrongly identified strain, DAOM 196850 (Chen *et al.* 2007), that is not a *Colletotrichum* species; its ITS sequence (GenBank EU400150) is identical to several *Plectosphaerella cucumerina* strains.

Colletotrichum pisicola is characterised by distinctly curved conidia that gradually taper to the ± acute ends, short and few pale brown setae with rounded tips. Strain CBS 724.97 is the slowest growing culture in the species complex studied.

The sequences of all loci studied of *C. pisicola* strain CBS 724.97 are unique; there is on CHS-1 only a single nucleotide difference to *Colletotrichum* sp. strain CBS 107.40 from *Pisum* in Russia (see *Colletotrichum* sp. CBS 107.40). No ITS sequences with >98 % identity (9 nucleotides different) and no TUB2 sequence with >91% identity were found in GenBank.

Colletotrichum sp. CBS 107.40

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–8 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata*, *conidiophores*, *conidiogenous cells* and *Setae* not observed. No sporulation. *Appressoria* single, scattered, pale brown, smooth-walled, ellipsoidal, clavate to navicular outline, with an entire or undulate margin, (4.5–)6.5–12(–15) × (3.5–)4.5–7.5(–9.5) µm, av. ± SD = 9.2 ± 2.7 × 5.9 ± 1.5 µm, L/W ratio = 1.6.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on pale to medium brown, roundish to angular cells, 4–11.5 µm diam. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae. *Setae* not observed. *Conidiophores* and *conidiogenous cells* not observed. *Conidia* only few observed, hyaline, smooth-walled, aseptate, ± curved, with slightly acute ends, 13–16.5 × 3.5–4 µm, mean = 14.9 × 3.8 µm, L/W ratio = 4.0.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline, agar medium, filter paper and *Anthriscus* stem partly covered with sparse aerial mycelium, reverse same colours; growth 12.5–14.5 mm in 7 d (18.5–20.5 mm in 10 d). Colonies on OA flat with entire margin; greenish olivaceous to citrine, with a straw margin, aerial mycelium lacking, reverse straw to greenish olivaceous, growth 17.5–19 mm in 7 d (25–28.5 mm in 10 d). *Conidia* in mass not visible.

Material examined: **Russia**, Omsk, from *Pisum sativum*, collection date and collector unknown (deposited in CBS collection Feb. 1940 by K. Murashkinsky), CBS H-21645, culture CBS 107.40.

Notes: Stain CBS 107.40 from peas in Russia was deposited as *Macrophoma sheldonii* in CBS by K. Murashkinsky. This species was described by Rodigin (1928) from seeds of *Pisum sativum* in Russia as forming cylindrical-ovate, thick-walled conidia, measuring 10–18 × 5–6 µm that are mass pink and formed in

spherical to flattened pycnidia. This species, if a *Colletotrichum* species at all, is not the same species as strain CBS 107.40, as conidial shapes and sizes are different. The spherical "pycnidia" could refer to the closed conidiomata that have been observed in species of the *C. boninense* species complex, *e.g. C. dacrycarpi* and *C. karstii* (Damm *et al.* 2012). *Macrophoma sheldonii* was regarded as a synonym of *C. lagenarium* by Vassiljevski & Karakulin (1950) and of *C. orbiculare* by von Arx (1957). Since we have not seen type material of this fungus, we cannot confirm this species as a *Colletotrichum* sp.

After the strain was deposited in CBS, it was re-identified as *C. pisi*. The strain was also treated as *C. pisi* by Nirenberg *et al.* (2002), who submitted an ITS sequence to GenBank (GenBank AJ301940). The conidia of strain CBS 107.40 are shorter than those of *C. pisicola* strain CBS 724.97, and more similar to *C. pisi* than those of *C. pisicola* (newly described in this study). However, we refrain from using this strain to epitypify *C. pisi*, because the strain is degenerated, the sporulation almost suppressed, and only four conidia were observed that might not be typical of the species. Moreover, the strain was from a different continent than *C. pisi*.

The sequences of all loci studied are unique for this species, and different from those of *C. pisicola* strain CBS 724.97; however, the CHS-1 sequence differs in only one nucleotide from that of *C. pisicola*.

There is no match with sequences >97 % identical to our ITS sequence and no match with sequences >89 % identical to our TUB2 sequence in GenBank.

Colletotrichum tabacum Böning, Prakt. Blätt. Pflanzenbau Pflanzenschutz 10: 89. 1932. Fig. 12.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–7 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae, sometimes also on few pale to medium brown, roundish cells, 5–10.5 µm diam. *Setae* pale to medium brown, smooth-walled, 65–150 µm long, 1–4-septate, base cylindrical to conical, 4–5.5 µm diam, tip ± rounded to ± acute. *Conidiophores* hyaline to pale brown, smooth-walled, septate, branched, to 50 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical to ampulliform, 9–22.5 × 3–4.5 µm, opening 1–1.5 µm diam, collarette 0.5–1 µm long, periclinal thickening observed. *Conidia* hyaline, smooth-walled, aseptate, narrowly cylindrical, mostly straight, with round ends, one of the ends sometimes very slightly bent to one side, (13.5–)15.5–18.5(–20) × 3–3.5(–4) µm, av. ± SD = 17.0 ± 1.4 × 3.4 ± 0.2 µm, L/W ratio = 5.0, conidia of strain CBS 124249 longer, measuring (16–)17–20(–23.5) × 3–3.5. *Appressoria* single or in loose groups, medium brown, smooth-walled, clavate, ellipsoidal or irregular outline, with a lobate to undulate margin, with a distinct penetration pore with a dark halo, (7–)8–13(–19) × (4.5–)5.5–8(–10) µm, av. ± SD = 10.4 ± 2.3 × 6.6 ± 1.3 µm, L/W ratio = 1.6.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on a small cushion of hyaline to pale brown, angular cells, 3–6 µm diam. *Setae* medium brown, smooth-walled to finely verruculose, 55–170 µm long, 1–5-septate, base cylindrical, 3.5–8.5 µm diam, tip ± rounded to ± acute. *Conidiophores* hyaline to pale brown, single or smooth-walled and septate, branched, to 30 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical to doliiform, 7–16.5 × 3.5–5 µm, opening 1–1.5 µm diam, collarette 0.5–1.5 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, narrowly cylindrical, mostly straight, with round ends, one of the ends sometimes very slightly bent to one side, (16.5–)17–19(–21) × (3–)3.5(–4) µm, av. ± SD = 17.8 ± 1.0 × 3.5 ± 0.2 µm, L/W ratio = 5.1.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline to cinnamon, aerial mycelium lacking, reverse same colours; growth 25–26.5 mm in 7 d (>40 mm in 10 d). Colonies on OA flat with entire margin; isabelline, honey, buff to rosy buff, aerial mycelium lacking, reverse buff, vinaceous buff, hazel to pale olivaceous-grey, colonies of strain CBS 124249 differed slightly on OA: colonies buff, almost entirely covered with honey, grey to black acervuli and partly covert with white short filty aerial mycelium, reverse buff, honey to olivaceous-grey, growth 27.5–29 mm in 7 d (>40 mm in 10 d). *Conidia* in mass salmon, conidia of strain CBS 161.53 in mass whitish.

Materials examined: **France**, from *Nicotiana tabacum*, collection date and collector unknown (received from R. O'Connell, before from P. Goodwin, before from M. Maurhofer Bringolf, originally from Novartis as Novartis Isolate 150) (CBS H-21669 **neotype here designated**, MBT178524, culture ex-neotype N150 = CPC 18945). **Germany**, Middle Franconia, from leaves of *Nicotiana rustica*, holotype, presumably lost. **India**, Rajahnundry, from *Nicotiana tabacum*, collection date unknown, B. S. Kadam, culture IMI 50187 = CPC 16820. **Madagascar**, from *Centella asiatica*, collection date and collector unknown (isolated by Rakotoniriana F. 2003), CBS H-21668, culture CBS 124249 = MUCL 44942. **Zambia**, from *Nicotiana tabacum*, collection date and collector unknown (send to CBS collection Nov. 1953 from Mt. Makulu Research St., Zambia), CBS H-21667, culture CBS 161.53.

Notes: In the late 1920s anthracnose of tobacco, especially *Nicotiana rustica*, was observed in Middle Franconia, Germany. The pathogen, *C. tabacum*, differed morphologically from the previously described *C. nicotianae* Averna (Böning 1929, 1932). The fungus formed conidia that measured 15–22 × 4–5 µm in small open clusters and setae that were 60–90 µm long (Böning 1929). In contrast, *C. nicotianae* that was described from stems of *N. tabacum* in Sao Paulo, Brazil, formed straight to curved conidia that were larger than those of *C. tabacum*, measuring 19–32.5 × 8–8.6 µm and turn yellow with age, and setae that were 60–175 × 8.5 µm long and 3–5-septate (Averna-Saccá 1922). *Colletotrichum tabacum* forms distinct spots with necrotic centres on leaves, stems, flowers and seeds and also causes a seedling disease of tobacco (Böning 1929). The microscopical features of the isolates studied here agree with *C. tabacum*, although the conidia are slightly smaller than those observed by Böning (1929). Böning (1929, 1932) did not designate a type, and no type or authentic material could be located in any fungarium.

Shortly after, an additional species was described by Böning (1933), *Gloeosporium nicotianae* that caused blisters and diffuse browning on leaf surfaces of *N. rustica* in Königsberg, East Prussia (today Kaliningrad, Russia), consistently lacked setae and also exhibited different cultural characteristics. *Colletotrichum tabacum* formed greenish black cultures with a uniform grey aerial mycelium vs. *Gm. nicotianae* with slightly brownish cultures and floccose aerial mycelium. Conidia of *Gm. nicotianae* are on average smaller than those of *C. tabacum*, measuring 8–18 × 2–5 µm, depending on the substrate and formed swollen, 12 µm diam cells in chains in the mycelium as well as sterile pycnidia- or perithecia-like structures (Böning 1933).

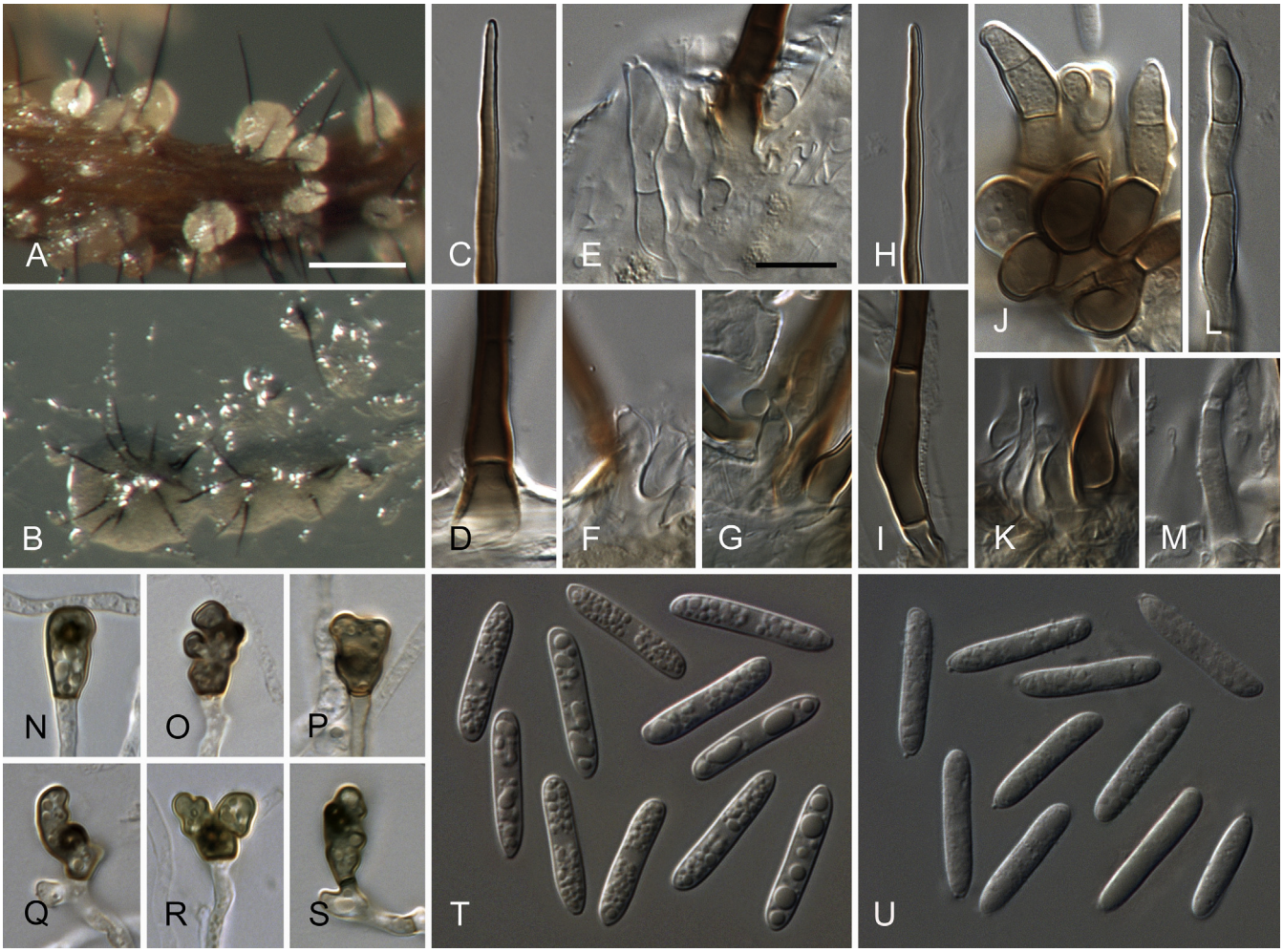

Fig. 12. *Colletotrichum tabacum* (from ex-neotype strain N150). A–B. Conidiomata. C, H. Tip of a seta. D, I. Base of a seta. E–G, J–M. Conidiophores. N–S. Appressoria. T–U. Conidia. A, C–G, T. from *Anthriscus* stem. B, H–S, U. from SNA. A–B. DM, C–U. DIC, Scale bars: A = 100 µm, E = 10 µm. Scale bar of A applies to A–B. Scale bar of E applies to C–U.

Based on the description alone it is difficult to confirm whether *C. tabacum* and *Gm. nicotianae* are different species.

Lucas & Shew (1991) concluded *C. nicotiniae* and *C. tabacum* were synonyms of *C. gloeosporioides*. This was probably based on von Arx (1957), who listed *C. tabacum* as synonym of *C. gloeosporioides*. Farr & Rossman (2014) cited various reports of *C. nicotianae*, *C. tabacum*, *C. destructivum*, *C. coccodes*, *C. gloeosporioides* and *Colletotrichum* sp. from tobacco around the world. One of the studies cited (Barksdale 1972) includes a picture and measurements of conidia of *C. destructivum* from tobacco that resemble those of *C. tabacum*. Isolates from *Nicotiana* used in molecular studies of pathogen-host-interactions are either called *C. nicotianae*, or *C. destructivum* (e.g. Chen *et al.* 2003; Yang *et al.* 2010). Based on rDNA ITS sequences and morphology, Shen *et al.* (2001) identified strain N150 (here re-identified as *C. tabacum*) as *C. destructivum*. As the isolates studied here were previously identified as *C. destructivum*, *C. higginsianum*, *C. gloeosporioides* or *C. tabaci*, many of the reports listed by Farr & Rossman (2014) might actually refer to *C. tabacum*. The few isolates of *C. tabacum* included in this study already represent the occurrence of the species on three continents. But to our knowledge, there is no report listed from Germany since Böning (1933).

Shen *et al.* (2001) discovered the intracellular hemibiotrophic infection process of *C. destructivum* (here re-identified as *C. tabacum*) strain N150 on tobacco. Shan & Goodwin (2004, 2005) used a GFP-expressing transgenic strain of this fungus to study rearrangement of host actin microfilaments and nuclei around biotrophic hyphae. Secondary metabolite production by *C. tabacum* (ATCC 11995) was extensively studied by Gohbara and co-workers during the 1970s, leading to the identification and structural characterisation of two novel terpenoid phytotoxins, colletotrichin and colletopyrone (Gohbara *et al.* 1976, 1978).

One of the strains included in this study, CBS 124249 (= MUCL 44942) was isolated by F. Rakotoniriana from *Centella asiatica* in Madagascar and identified as *C. higginsianum* (Rakotoniriana *et al.* 2008). It is re-identified as *C. tabacum* in this study. Rakotoniriana *et al.* (2013) recently described a species from *Centella asiatica* in Madagascar, *C. gigasporum* that forms larger conidia than *C. tabacum* and belongs to the *C. gigasporum* complex (Liu *et al.* 2014), confirming that more *Colletotrichum* species occur on this host in Madagascar.

Conidia of *C. tabacum* are narrowly cylindrical with round ends, one of the ends sometimes slightly bent to one side; the conidia still appearing straight. Appressoria with a distinct penetration pore with a dark halo were observed.

Colletotrichum tabacum is distinguished from the other species in the *C. destructivum* complex by all loci studied, but sequences of some loci only differ with a single nucleotide from its closest relative. Strain CBS 124249 from *Centella* differs additionally in CHS-1 and TUB2 sequences from the other three

strains, but intraspecific variability was also observed with ITS, GAPDH and ACT.

The closest match in a blastn search with the TUB2 sequence of strain N150 with 100 % identity was *C. tabacum* strain CBS 161.53 (GenBank JQ005847, O'Connell *et al.* 2012). No GAPDH sequence with <93 % identity was found in GenBank.

Colletotrichum tanaceti M. Barimani, *et al.*, Plant Pathol. 62: 1252. 2013. Fig. 13.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–10 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae. *Setae* medium brown, smooth-walled to verruculose, 40–140 µm long, 2–4-septate, base cylindrical to conical, 4–5.5 µm diam, tip rounded to slightly acute. *Conidiophores*, smooth-walled, septate, branched, to 75 µm long. *Conidiogenous cells* hyaline, smooth-walled, sometimes extending to form new conidiogenous loci, 15–28 × 3.5–4.5 µm, opening 1–2 µm diam, collarette 1 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, cylindrical to slightly clavate, slightly but distinctly curved with both ends ± rounded, (13–)14.5–17.5(–19) × (3–)3.5–4(–4.5) µm, av. ± SD = 16.0 ± 1.5 × 3.8 ± 0.3 µm, L/W ratio = 4.2. *Appressoria* single or in loose groups, medium brown, smooth-walled, subglobose, to elliptical in outline, with an entire or undulate margin, (5–)6.5–12(–14.6) × (3.5–)4.5–7(–10) µm, av. ± SD = 9.1 ± 2.7 × 5.7 ± 1.4 µm, L/W ratio = 1.6, appressoria of stem CBS 132818 are slightly larger, measuring (7.5–)8.5–13.5(–16) × (5–)5.5–9(–12) µm, av. ± SD = 11.0 ± 2.5 × 7.4 ± 1.8 µm, L/W ratio = 1.5.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on hyaline to pale brown, angular cells, 3–7.5 µm diam. *Setae* medium brown, smooth-walled to finely verruculose, 30–165 µm long, 1–4-septate, base cylindrical to conical, 4–7 µm diam, tip rounded to slightly acute. *Conidiophores* hyaline to pale brown, smooth-walled, septate, branched, to 50 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical, sometimes extending to form new conidiogenous loci, 18–28 × 4–5 µm, opening 1–1.5 µm diam, collarette 0.5 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, slightly but distinctly curved with both ends ± rounded or one end ± acute, (12–)16–20.5(–22) × (3–)3.5–4(–4.5) µm, av. ± SD = 18.1 ± 2.1 × 3.7 ± 0.3 µm, L/W ratio = 4.9.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline to pale isabelline, filter paper partly yellow, aerial mycelium lacking, reverse same colours; growth 14–16 mm in 7 d (22.5–25 mm in 10 d). Colonies on OA flat with entire margin, buff to straw, partly covered with tiny grey to black acervuli, aerial

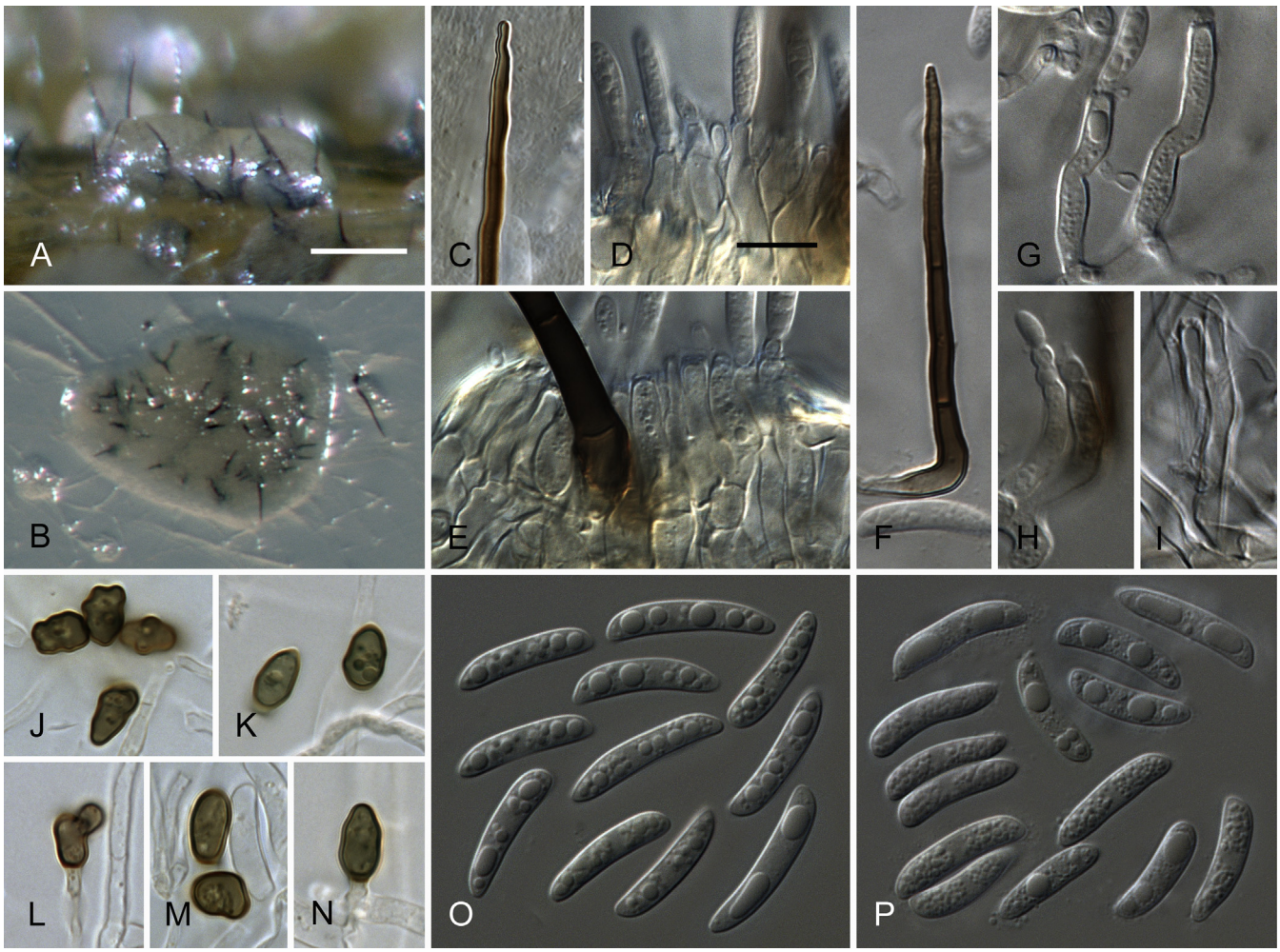

Fig. 13. *Colletotrichum tanaceti* (from ex-holotype strain CBS 132693). A–B. Conidiomata. C. Tip of a seta. D, G–I. Conidiophores. E. Base of a seta and conidiophores. F. Seta. J–N. Appressoria. O–P. Conidia. A, C–E, O. from *Anthriscus* stem. B, F–N, P. from SNA. A–B. DM, C–P. DIC, Scale bars: A = 100 µm, D = 10 µm. Scale bar of A applies to A–B. Scale bar of D applies to C–P.

mycelium lacking, reverse olivaceous-grey, growth 14.5–17 mm in 7 d (21.5–24.5 mm in 10 d). *Conidia* in mass whitish to rosy-buff.

Materials examined: **Australia**, northern Tasmania, Scottsdale, from anthracnose on leaves of *Tanacetum cinerariifolium*, Aug. 2010, S.J. Pethybridge, **culture ex-holotype** CBS 132693 = BRIP 57314 = UM01; Australia, northern Tasmania, Ulverstone, from *Tanacetum cinerariifolium*, collection date unknown, S.J. Pethybridge, living strain CBS 132818 = BRIP 57315 = TAS060-0003.

Notes: Pyrethrum (*Tanacetum cinerariifolium*, *Asteraceae*) is a perennial plant grown for the extraction of pyrethrin insecticides in Australia, mainly in Tasmania, one of the largest producers of pyrethrin worldwide (Greenhill 2007). *Colletotrichum tanaceti* was recently described as an anthracnose pathogen of pyrethrum in Tasmania and revealed to be closely related to *C. destructivum*, *C. higginsianum* and *C. panacicola* (Barimani *et al.* 2013). This species can be confirmed as distinct in this study, and can be identified with all loci studied.

Additionally, *C. tanaceti* is one of the two species in this complex with distinctly curved conidia. In contrast to *C. pisicola*, the conidia are more abruptly tapered towards mostly rounded ends. In both media, conidiogenous cells were observed that extended to form new conidiogenous loci (Fig. 14E, H), a feature common for species in the *C. boninense* species complex (Damm *et al.* 2012) but not elsewhere in the *C. destructivum* complex.

Our conidia measurements differ from those given in the study of Barimani *et al.* (2013). In that study, conidia on pyrethrum tissue measured on average 30.9 × 5.6 µm, and those on SNA, 22.5 × 4.1 µm. In contrast, conidia of the same strain on SNA measured in our study on average 16.0 × 3.8 µm.

This fungus formed perithecia in a mating experiment and is apparently heterothallic (Barimani *et al.* 2013). The sexual morph was described by Barimani *et al.* (2013) as follows "Perithecia dark brown, ampulliform with setaceous hairs in ostiole, becoming erumpent through the epidermis, perithecia ostiolate measuring 33 × 31 µm in diameter, individual locules measuring 200 × 380 µm (length × width), thick-walled texture. Asci 89.6 ± 2.9 × 10.9 ± 0.4 µm (n = 30), unitunicate, thin-walled, clavate or cymbiform, stipitate, 8–10 spored. Ascospores (18–)21.5–22.5(–26.5) × (4–)5.5–6(–7) µm (n > 50), av. ± SD = 22 ± 1.7 × 5.8 ± 0.7 µm, one-celled, hyaline, smooth, becoming septate through germination, fusiform and blunt at both ends (widest at middle and narrower at the ends) or widest at middle and upper third, many formed within 2 months."

Barimani *et al.* (2013) also studied the infection strategy, which they suggested to be intracellular hemibiotrophic, similar to that of *C. destructivum* and *C. higginsianum*.

Colletotrichum utrechtense Damm, **sp. nov.** MycoBank MB809404. Fig. 14.

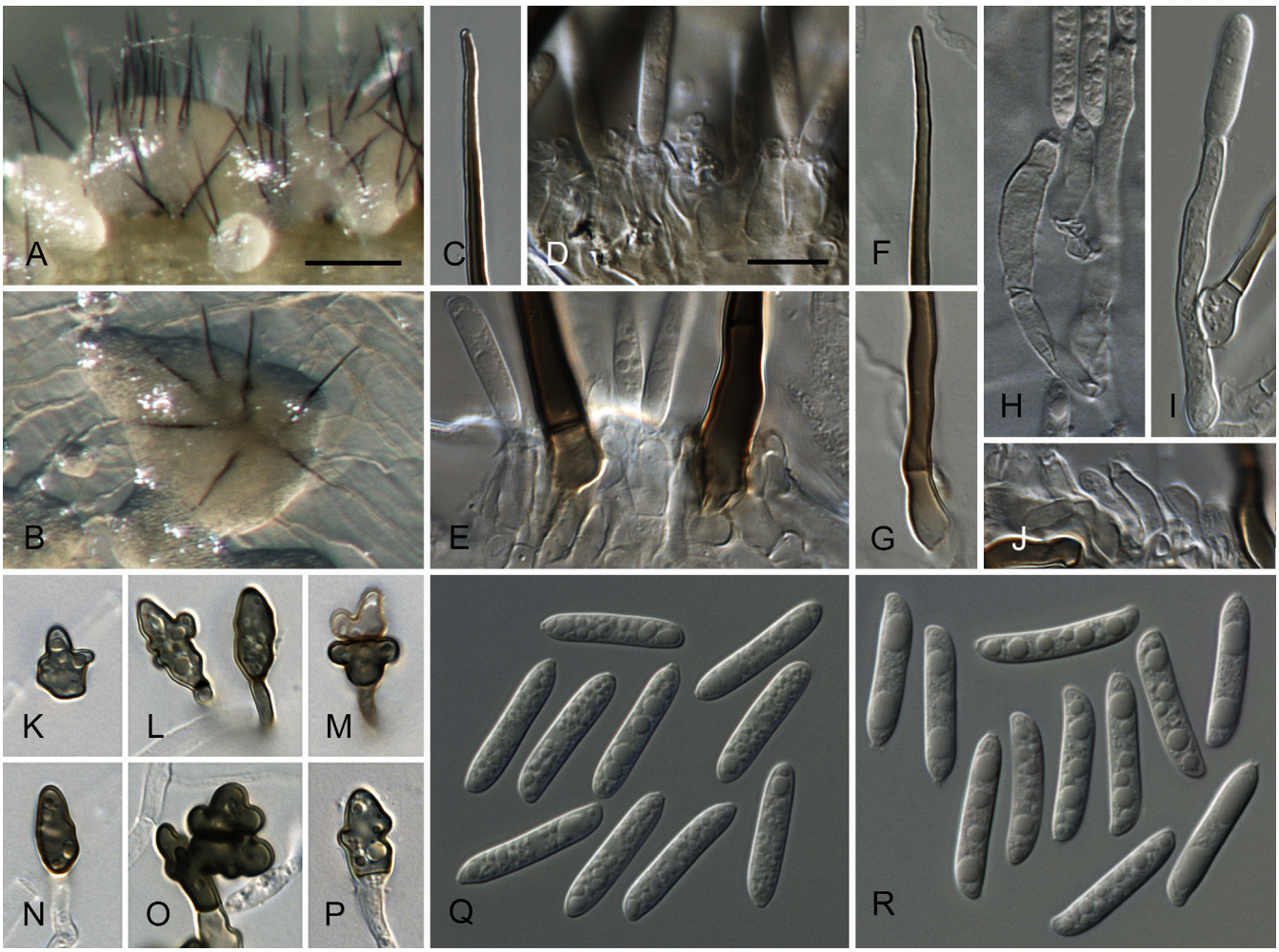

Fig. 14. *Colletotrichum utrechtense* (from ex-holotype strain CBS 130243). A–B. Conidiomata. C, F. Tip of a seta. D, H–J. Conidiophores. E. Bases of setae and conidiophores. G. Base of a seta. K–P. Appressoria. Q–R. Conidia. A, C–E, Q. from *Anthriscus* stem. B, F–P, R. from SNA. A–B. DM, C–R. DIC, Scale bars: A = 100 µm, D = 10 µm. Scale bar of A applies to A–B. Scale bar of D applies to C–R.

Etymology: The species epithet is derived from the place where it was collected, Utrecht, the Netherlands.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–7.5 µm diam, hyaline to pale brown, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae. *Setae* medium brown, smooth-walled to finely verruculose, 95–180 µm long, 2–5-septate, base cylindrical to ± inflated, 3–6.5 µm diam, tip ± rounded to slightly acute. *Conidiophores* hyaline to pale brown, smooth-walled, septate, branched, to 70 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical to ± inflated, 13–26 × 3–4.5 µm, opening 1–1.5 µm diam, collarette 1–1.5 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, straight to slightly curved, with both ends ± rounded, 17.5–20.5(–23) × 3.5–4(–4.5) µm, av. ± SD = 19.0 ± 1.4 × 4.0 ± 0.2 µm, L/W ratio = 4.8. *Appressoria* single, sometimes in clusters of two, medium brown, smooth-walled, navicular, ellipsoidal or irregular in outline, with an lobate or undulate margin, (7–)10–14.5(–15) × (5–)6.5–9.5(–10) µm, av. ± SD = 12.2 ± 2.1 × 8.0 ± 1.5 µm, L/W ratio = 1.5, appressoria of strain CBS 135827 smaller, measuring (6.5–)7.5–13.5(–19) × (3.5–)4.5–7(–9) µm, av. ± SD = 10.5 ± 3.0 × 5.7 ± 1.3 L/W µm, ratio = 1.8.

Asexual morph on Anthriscus stem. Conidiomata absent, conidiophores and setae formed directly on hyphae, or rarely on pale brown, angular cells, 3.5–8 µm diam. *Setae* medium brown, basal cell pale brown, smooth-walled to finely verruculose, 75–255 µm long, 2–4-septate, base ± inflated or cylindrical, 3.5–8.5 µm diam, tip slightly rounded to slightly acute. *Conidiophores* hyaline to pale brown, smooth-walled, simple or septate and branched, to 20 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, ellipsoidal to cylindrical, 9–17 × 4–5.5 µm, opening 1–2 µm diam, collarette 1–2 µm long, periclinal thickening distinct. *Conidia* hyaline, smooth-walled, aseptate, straight to slightly curved, with both ends ± rounded, (16.5–)18–20(–21.5) × 3.5–4 µm, av. ± SD = 19.0 ± 1.0 × 3.7 ± 0.2 µm, L/W ratio = 5.2.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline, pale cinnamon in the centre, filter paper partly pale olivaceous grey, *Anthriscus* stem partly covert with filty white aerial mycelium, reverse same colours; 20–24 mm in 7 d (35–36.5 mm in 10 d). Colonies on OA flat with entire margin; buff, pale cinnamon, pale olivaceous grey to olivaceous grey, with few patches of floccose, whitish, aerial mycelium, reverse same colours, 22.5–25 mm in 7 d (33.5–39 mm in 10 d). *Conidia* in mass whitish to very pale salmon.

Materials examined: **Netherlands**, Utrecht, from a leaf of *Trifolium pratense,* 13 Jun. 2011, U. Damm (CBS H-21662 **holotype**, culture ex-holotype CBS 130243); Utrecht, from a leaf of *T. pratense,* 13 Jun. 2011, U. Damm, culture CBS 135827; Utrecht, from a leaf of *T. pratense,* 13 Jun. 2011, U. Damm, culture CBS 135828.

Notes: This species is only known from *Trifolium pratense* in the Netherlands. Other *Colletotrichum* species described from this host are reviewed in the notes under *C. destructivum*.

The CHS-1, HIS3 and TUB2 sequences are different from all species included. The ACT sequences are the same as that of *C. panacicola*; ITS and GAPDH distinguishes the species from *C. panacicola* but the ITS is identical with the unnamed isolates from *Heracleum*, while the GAPDH sequence is the same as that of *C. higginsianum* and the isolates from *Heracleum* and *Matthiola.*

In blastn searches the ITS and GAPDH sequences of strain CBS 130243 were found to be identical to the ITS sequence of "*C. coccodes*" strain BBA 71527 from *Lupinus* in Germany (GenBank AJ301984, Nirenberg *et al.* 2002) and the GAPDH sequences of *C. higginsianum* isolates C97027 and C97031 from *Brassica* and *Raphanus* probably from Korea (GenBank GU935850, GU935851, Choi *et al.* 2011). Closest matches in blastn searches with the TUB2 sequences of strain CBS 130243 with 99 % identity (3 nucleotides different) were *C. fuscum* CBS 130.57 (GenBank JQ005846, O'Connell *et al.* 2012) and *Colletotrichum* isolates from a study on ramie (*Boehmeria nivea*) anthracnose in China (GenBank JF811024–JF811028, W.X. Xia, unpubl. data).

Colletotrichum vignae Damm, **sp. nov.** MycoBank MB809405. Fig. 15.

Etymology: The species epithet is derived from the host genus name *Vigna*.

Sexual morph not observed. *Asexual morph on SNA. Vegetative hyphae* 1–8 µm diam, hyaline, smooth-walled, septate, branched. *Chlamydospores* not observed. *Conidiomata* absent, conidiophores and setae formed directly on hyphae. *Setae* hyaline to very pale brown, smooth-walled, wall up to 0.8 µm wide, 30–90 µm long, 1–3-septate, base cylindrical to conical, 3–4.5 µm diam, tip rounded to ± acute. *Conidiophores* hyaline, sometimes pale brown, smooth-walled, septate, branched, to 35 µm long. *Conidiogenous cells* hyaline, sometimes pale brown, smooth-walled, cylindrical, 12–25 × 3–5 µm, polyphialides observed, opening 1–1.5 µm diam, collarette 0.5–2 µm long, periclinal thickening sometimes observed. *Conidia* hyaline, smooth-walled, aseptate, old conidia sometimes septate, cylindrical, straight to slightly curved, with one end round and the other truncate, (12–)14–17.5(–18.5) × (3–)3.5–4(–4.5) µm, av. ± SD = 15.8 ± 1.6 × 3.8 ± 0.3 µm, L/W ratio = 4.2. *Appressoria* not observed on the undersurface of the medium. Appressoria-like structures that possibly function as chlamydospores were observed within the medium. These are single or in dense clusters, medium brown, smooth-walled, ellipsoidal, subglobose to clavate outline, with an entire or undulate margin, because not attached to any surface (4–)4.5–8.5(–12.4) × (3.5–)4–5(–6.5) µm, av. ± SD = 6.6 ± 2.0 × 4.6 ± 0.6 µm, L/W ratio = 1.4.

Asexual morph on Anthriscus stem. Conidiomata, conidiophores and setae formed on pale brown, angular cells, 2.5–8 µm diam. *Setae* pale to medium brown, smooth-walled to verruculose, very thick-walled (up to 1.5 µm wide), 40–120 µm long, 1–3-septate, base conical to cylindrical, 5–8.5 µm diam, tip rounded to slightly acute. *Conidiophores* hyaline, smooth-walled, septate, branched, to 60 µm long. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical, 8–35 × 3.5–4(–6.5) µm, opening 1–1.5 µm diam, collarette 0.5–1 µm long, periclinal thickening visible. *Conidia* hyaline, smooth-walled, aseptate, cylindrical, straight to slightly curved, with one end round to slightly acute and the other truncate, (10–)12–16.5(–21.5) × 3.5–4 µm, av. ± SD = 14.2 ± 2.2 × 3.8 ± 0.2 µm, L/W ratio = 3.8. conidia of strain IMI 334960 are shorter, measuring (8–)9–13(–15.5) × (3.5–)4–4.5(–5) µm, av. ± SD = 11.0 ± 1.8 × 4.3 ± 0.3 µm, L/W ratio = 2.6.

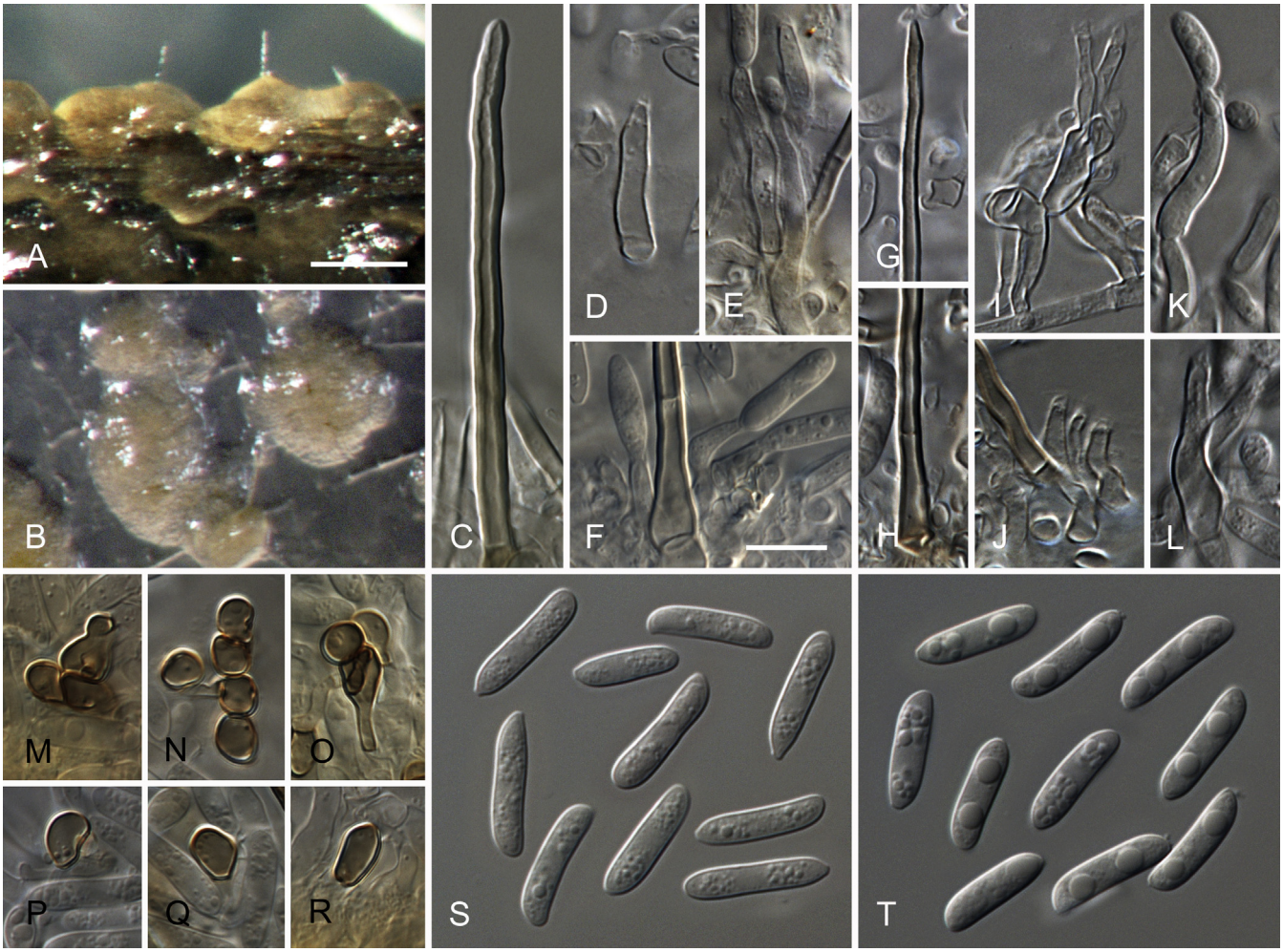

Fig. 15. *Colletotrichum vignae* (from ex-holotype strain CBS 501.97). A–B. Conidiomata. C, G. Tip of a seta. D–E. Conidiophores. F. Base of a seta and conidiophores. H. Base of a seta. I–L. Conidiophores. M–R. Appressorium-like structures. S–T. Conidia. A, C–F, S. from *Anthriscus* stem. B, G–R, T. from SNA. A–B. DM, C–T. DIC, Scale bars: A = 100 µm, F = 10 µm. Scale bar of A applies to A–B. Scale bar of F applies to C–T.

Culture characteristics: Colonies on SNA flat with entire margin, hyaline, agar medium, filter paper and *Anthriscus* stem partly covered with saffron to cinnamon acervuli, aerial mycelium lacking, reverse same colours; growth 12.5–15 mm in 7 d (19–22.5 mm in 10 d). Colonies on OA flat with entire margin; honey to cinnamon, with a buff margin, aerial mycelium lacking, reverse same colours; growth 13.5–15 mm in 7 d (20–21.5 mm in 10 d). Colours and growth rate of strain IMI 334960 differed on OA by being dark grey olivaceous, partly covered with short white aerial mycelium, reveres dark grey olivaceous to olivaceous grey; growth 17–18.5 mm in 7 d (24.5–26 mm in 10 d). *Conidia* in mass saffron.

Materials examined: **Nigeria**, from *Vigna unguiculata*, collection date unknown (deposited in CBS collection Feb. 1997 by J.A. Bailey, isolated by R.A. Skipp, No. I 57), R.A. Skipp (CBS H-21648 **holotype**, culture ex-type CBS 501.97 = LARS 56); from *Vigna unguiculata*, collection date and collector unknown, culture IMI 334960 = CPC 19383.

Notes: The isolates studied here apparently originate from a study on cowpea diseases in Nigeria by Williams (1975), who sent isolates to IMI where they were identified as *C. lindemuthianum.* In contrast to the species studied here, *C. lindemuthianum* belongs to the *C. orbiculare* species complex (Damm *et al.* 2013, Liu *et al.* 2013a). Judging from information retrieved from Bailey *et al.* (1990) these two strains originated from the same isolate.

Bailey *et al.* (1990) observed the single-cell hemibiotrophic infection of cowpea by *C. lindemuthianum* from cowpea (= *C. vignae*) for the first time. They also revealed the morphology, pathogenicity and host specificity of strain I57 (= LARS 56 = CBS 501.97) to be different from *C. lindemuthianum* isolates from *Phaseolus vulgaris*. Latunde-Dada *et al.* (1996, 1999) studied the infection of cowpea by the same strain and by strain LARS 860, another strain from cowpea in Nigeria. They identified the species as *C. destructivum* based on similarity of morphological features and the ITS2-D2 sequences with isolates from *Medicago* that were confirmed as *C. destructivum s. str.* in our study. However, based on the ITS2-D2 phylogeny of the second study strain, LARS 860 is a different species to LARS 56 (*C. vignae*), not belonging to the *C. destructivum* complex and more closely related to *C. gloeosporioides* (*s. lat.*) strains; the infection process differs considerably and is not hemibiotrophic.

Takimoto (1934) described *C. phaseolorum* from *Vigna angularis* and *V. sinensis* in Japan. The type of this species was not designated. Authentic isolates from the two hosts studied by Damm *et al.* (2009) are not conspecific, but neither is closely related to *C. vignae*. In contrast to *C. vignae*, this species forms distinctly curved conidia. Further isolates from *Vigna*, from *V. unguiculata* in Burkina Faso and *V. sinensis* in Pakistan, respectively, were recently identified as *C. truncatum* in the same multilocus analyses (Damm *et al.* 2009). *Colletotrichum phaseolorum* was treated as a synonym of *C. gloeosporioides* by

von Arx (1957, wrongly cited as *C. phascorum*). Shen *et al.* (2010) reported anthracnose of mung bean (*V. radiata*) sprouts to be caused by *C. acutatum* (*s. lat.*) in Taiwan.

Glomerella vignicaulis was described by Tehon (1937) on *Vigna sinensis* in Illinois, USA. Tehon never found an asexual *Colletotrichum* morph on the host; however, a *Cercospora* stage accompanying the perithecia that appeared to arise from the same mycelium was always observed. If *Ga. vignicaulis* is a *Colletotrichum* species at all, it is unlikely to belong to the *C. destructivum* species complex, as in this complex conidia are dominating; none of the species is known to form a sexual morph in nature. The two species that are known to form a sexual stage, *C. lentis* (= *Ga. truncata*) and *C. tanaceti*, are apparently heterothallic and sexual morphs were only observed by crossing experiments in the laboratory (Armstrong-Cho & Banniza 2006, Barimani *et al.* 2013).

Colletotrichum vignae was one of the slowest growing species studied in the *C. destructivum* complex and the slowest growing species within the first main clade (Fig. 1). Conidia of *C. vignae* are highly variable in length; the few setae observed were pale brown and thick-walled.

This species can be identified by its ITS, GAPDH, HIS3 and ACT sequences. Blastn searches with the respective sequences of strain CBS 501.97 resulted in 99 % identity (a single nucleotide difference) with the ITS sequence of *C. fuscum* strain DAOM 216112 (GenBank EU400144; Chen *et al.* 2007), and 98 % identity with the GAPDH sequences of *C. higginsianum* isolates C97027 and C97031 from *Brassica* and Raphanus, respectively, probably from Korea (GenBank GU935870 and GU935873; Choi *et al.* 2011), and 99 % identity (2 nucleotides difference) with the HIS3 sequence of *C. higginsianum* isolate MAFF 305635 (GenBank JQ005803; O'Connell *et al.* 2012, included in this study) and 99 % identity (a single nucleotide difference) with the ACT sequences of *C. fuscum* CBS 130.57 (GenBank JQ005825; O'Connell *et al.* 2012, included in this study), respectively. The CHS-1 sequences are the same as those of *C. fuscum*, *C. higginsianum*, *C. antirrhinicola* and the unnamed isolates from *Heracleum* and *Matthiola*.

Sun & Zhang (2009) isolated *Colletotrichum* from anthracnose lesions on leaves of cowpea in China that they identified as *C. destructivum* based on morphology. As the ITS sequences were the same as those from cruciferous hosts, they concluded *C. higginsianum* to be a synonym of *C. destructivum*. The ITS sequence from those strains, however, differed in 4 nucleotides from those of *C. vignae*.

DISCUSSION

Previous multilocus phylogenies have shown the *C. destructivum* species complex was monophyletic, and sister to the combined *C. graminicola* and *C. spaethianum* complexes (Cannon *et al.* 2012, O'Connell *et al.* 2012). Based on a multilocus phylogeny including a large number of isolates from various host plants, we differentiated several distinct species.

While, *C. destructivum*, *C. lini* and *C. fuscum* are regarded as separate species, von Arx (1957) listed *C. higginsianum* and *C. tabacum* as synonyms of *C. gloeosporioides*. However, these species are not closely related to *C. gloeosporioides* that belongs to a different species complex within the genus, and all five were regarded as distinct species in this study.

One characteristic morphological feature of the *C. destructivum* species complex is the conidia that are slightly curved due to their unilaterally tapering ends, which is apparent in most of the species. However, some species are distinctly curved (*C. pisicola, C. tanaceti*), while others are almost straight (especially *C. tabacum* and *C. ocimi*) and reminiscent of *C. coccodes* or *C. gloeosporioides*. The variation between almost straight and curved conidia in this species complex was one of the reasons for some isolates having been confused with species belonging to other species complexes, *e.g. Ga. glycines*, *C. coccodes*, *C. truncatum*, *C. gloeosporioides*, *C. lindemuthianum* or *C. trifolii*. Another typical characteristic is the small inconspicuous acervuli with rather effuse growth that are sometimes difficult to spot on the host plant. Latunde-Dada & Lucas (2007) observed several species in the *C. destructivum* complex that formed acervuli with only a single seta on the host plant. Setae are comparatively short, pale to medium brown, often smooth-walled with round apices. However, these features are variable on different culture media and large distinct acervuli with abundant dark setae may be produced as well, depending on species, strain, substrate and age of the culture. The size of conidia and appressoria is also variable within species, and usually not taxonomically informative for species differentiation.

Sexual morphs were not observed in the cultures used in this study. As far as we know, *C. destructivum s. str.* does not form a sexual morph. The sexual morph linked to it, *Ga. glycines*, is not closely related to *C. destructivum* and belongs to a different species complex (U. Damm, unpublished results). However, there are two heterothallic species, *C. lentis* (as *Ga. truncatum* by Armstrong-Cho & Banniza 2006) and *C. tanaceti* (Barimani *et al.* 2013) that form sexual morphs by artificially crossing isolates. In contrast, many species in the *C. boninense* species complex are apparently homothallic (Damm *et al.* 2012).

The most intensively-studied species in this complex are all serious economic pathogens. The infection strategy of several of them has been found to be hemibiotrophic. Using light and electron microscopy, O'Connell *et al.* (1993), Bailey *et al.* (1990) and Latunde-Dada *et al.* (1996, 1997) investigated the hemibiotrophic infection of *Pisum*, *Vigna* and *Medicago*, respectively, by isolates that are shown here to belong to three different species of the *C. destructivum* complex, namely *C. pisicola*, *C. vignae* and *C. destructivum s. str.* The infection processes of *C. lini* on flax (Hahn 1952), *C. tabacum* on tobacco (Shen *et al.* 2001), *C. higginsianum* on *Arabidopsis* (O'Connell *et al.* 2004), *Ga. truncata* (re-identified here as *C. lentis*) on lentil (Armstrong-Cho *et al.* 2012) and *C. tanaceti* on *Tanacetum* (Barimani *et al.* 2013) were very similar. The characteristic feature of hemibiotrophy in all these species is that initial penetration of the fungus by appressoria is followed by an intracellular biotrophic phase associated with fat, bulbous primary hyphae that invaginate the plasma membrane of living plant cells. Both the primary hyphae and the entire biotrophic phase are confined within a single epidermal cell. Much thinner, filamentous secondary hyphae then develop from the tips of the primary hyphae to rapidly colonise surrounding tissues. This morphological transition is associated with a switch to destructive necrotrophy and the appearance of disease symptoms. The major difference in all other hemibiotrophic *Colletotrichum* species so far examined (*e.g.* pathogens from the *C. orbiculare* and *C. graminicola* complexes) is that the primary hyphae are less bulbous and the biotrophic phase extends into many host cells (O'Connell *et al.* 1985, Wharton *et al.* 2001, Crouch *et al.* 2014). Probably all species are hemibiotrophic in the *C. destructivum* complex, but this needs confirmation.

Latunde-Dada & Lucas (2007) found a close relationship among isolates of several species in the *C. destructivum* complex. They also demonstrated that there are three clades within the genus *Colletotrichum* containing hemibiotrophic species, which they called *C. orbiculare*, *C. destructivum-linicola-truncatum* (including wrongly identified *C. truncatum* strains) and *C. cereale-graminicola-sublineolum* aggregates. Previously, hemibiotrophic *C. truncatum* isolates from different hosts, *e.g. Pisum* and *Lens*, were wrongly identified. These isolates belong to species in the *C. destructivum* complex. In contrast, *C. truncatum* (= *C. capsici*) is a different species that does not belong to this complex (Cannon *et al.* 2012) and utilises an infection strategy that is necrotrophic rather than hemibiotrophic (Pring *et al.* 1995). More recent studies on the infection process of *C. truncatum* on chili leaves and fruits using light microscopy (Ranathunge *et al.* 2012) and fluorescence microscopy of transformants expressing GFP (Auyong *et al.* 2012) revealed that an initial subcuticular-intramural endophytic phase was followed by a destructive, necrotrophic phase of colonisation.

Based on the host origins of species for which a large number of isolates were available, some species appear to be specific to certain genera or families of herbaceous plants, for example *C. fuscum* on *Digitalis* and *C. higginsianum* on *Brassicaceae* (Fig. 1). In contrast, other species appear to be generalists with broad host ranges, having been collected from taxonomically highly divergent plant families, *e.g. C. destructivum* from *Asteraceae, Fabaceae* and *Polygonaceae*, and notably *C. lini* from *Asteraceae, Brassicaceae, Fabaceae, Lamiaceae, Linaceae* and *Ranunculaceae*. In contrast, most species in the *C. graminicola* complex were restricted to single host species or genera (Crouch *et al.* 2009, Crouch 2014). Furthermore, we found that several host species can be attacked by more than one member of the *C. destructivum* complex. For example, *Medicago* and *Trifolium* are each attacked by three different species, while *Raphanus* and *Pisum* are each attacked by two different species (Fig. 1). There is much evidence that pathogen host range is determined by rapidly evolving secreted effector proteins that facilitate infection, notably by suppressing plant immunity (Schulze-Lefert & Panstruga 2011). Comparative genomic analyses of the effector repertoires of "specialist" and "generalist" members of the *C. destructivum* complex could thus provide important insights into the molecular basis of host range within this fungal clade.

Host range has been considered an unambiguous criterion for delimiting two species (for example Sun & Zhang, 2009). However, the results of pathogenicity tests with species from the *C. destructivum* complex are often contradictory. In laboratory assays with *C. higginsianum*, Higgins (1917) observed abundant infection of turnip (*Brassica rapa*) and radish (*Raphanus sativus*), limited leaf spotting on cabbage (*Brassica oleracea* capitata) and collards (*Brassica oleracea* viridis) and no infection of lettuce (*Lactuca sativa*). Sun & Zhang (2009) found that *C. higginsianum* isolates from cowpea (*Vigna unguiculata*) infected *Arabidopsis thaliana* and some cowpea cultivars, while other cowpea cultivars, lentil (*Lens culinaris*), Chinese cabbage (*Brassica rapa* subsp. *pekinensis*), and tobacco (*Nicotiana tabacum*) were all resistant. In pathogenicity tests by O'Connell *et al.* (2004), legume isolates of *C. destructivum* were unable to infect *A. thaliana,* while *C. destructivum* strain N150 (re-identified as *C. tabacum* in this study) infected tobacco, alfalfa (*Medicago sativa*), cowpea and *Medicago truncatula*, but not soybean (*Glycine max*) (Shen *et al.* 2001). In contrast, Manandhar *et al.* (1986) regarded *C. destructivum* as a soybean pathogen.

The contradictory results obtained from pathogenicity tests may be partly attributed to variation in factors affecting the host-pathogen interaction, for example incubation conditions (humidity and temperature), and variation in the inoculation methods used, such as detached leaves or intact host tissues. For example, Liu *et al.* (2007) found that *C. lini* could infect detached *Arabidopsis* leaves but not intact plants, due to senescence of the detached tissues, associated with impairment of salicylic acid- and ethylene/jasmonate-dependent host defense responses. A further problem is that isolates are frequently misidentified or only identified to species complex level. This likely explains the different results of pathogenicity tests obtained with *C. destructivum* (*s. lat.*) isolates from cowpea. The isolates from cowpea tested by Sun & Zhang (2009) have ITS sequences that are identical to *C. higginsianum*, while the isolate from cowpea included in the study by O'Connell *et al.* (2004) is a different species and described as *C. vignae* in this study (see notes under *C. vignae*). Moreover, fungus-host relationships can also be endophytic in nature. Thus, many *Colletotrichum* species were isolated as symptomless endophytes, including species of the *C. destructivum* complex from *Holcus* (Sánchez Márquez *et al.* 2012) and *Arabidopsis* (Garcia *et al.* 2013) in Spain and from *Rumex* (Hu *et al.* 2012) and *Bletilla* (Tao *et al.* 2013) in China. In conclusion, host range and pathogenicity can only provide indications of the identity of a *Colletotrichum* species, and should not be used as criteria for species delimitation or identification.

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