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

U. Damm1*, R.J. O’Connell2, J.Z. Groenewald1, and P.W. Crous1,3,4

1CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands; 2UMR1290 BIOGER-CPP, INRA-AgroParisTech, 78850 Thiverval-Grignon, France; 3Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa; 4Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

*Correspondence: U. Damm; ulrike.damm@senckenberg.de, Present address: Senckenberg Museum of Natural History Görlitz, PF 300 154, 02806 Görlitz, Germany.

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. Several *Colletotrichum* isolates, previously reported as closely related to *C. destructivum*, are known to cause 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 The inconsistent application of names to those isolates based on outdated species concepts has caused much taxonomic confusion, particularly in the plant pathology literature.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 *Ascomycota*. As a result, many previously identified species are newly described.

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

Taxonomic novelties: New species: *Colletotrichum americanum-borealis* Damm, *C. antirrhinicum* Damm, *C. bryonicola* Damm, *C. coccodes* Damm, *C. corymbosum* Damm; Typifications: *Epitypifications* (basionyms): *C. destructivum O’Gara*, *C. fuscum* Laubert, *C. higginsianum* Sacc., *C. lini* Westerd.; Lectotypifications (basionyms): *C. fuscum* Laubert, *G. lini* Westerd., *C. pisicola* Pat; Neotypification (basionym): *C. tabacum* Böning.

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 (e.g., *Chrysanthemum*, *Convolvulaceae* (*Cuscuta*), *Magnoliaceae* (*Michelia*), *Menispermatceae* (*Cocculus*), *Polygonaceae* (*Rumex*), *Solanaceae* (*Nicotiana*), *Lamiaceae* (*Perilla*), *Scrophulariaceae* (*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, 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. lini* 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. lini* isolates from *Linum* and *C. truncatum* isolates from *Plasm salivaria*, *Vicia faba*...
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. liniola* and *G. 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 C. destructivum only a few isolates were included in those studies. Huser et al. 2001) and several genes involved in plant infection have been studied (O’Connell et al. 2012) compared genome and transcriptome sequence data of *C. higginsianum* with those of *C. graminicola*, a hemibiotic 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.

Colonial 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 MgCl2, 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 Bioedit v. 4.60 (Applied Maths, St-Martens-Latem, 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 method.
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     |       |       |      |     |      |
|                          | CBS 136855     | Medicago sativa | USA         | KM105225     |       |       |      |     |      |
|                          | ATCC 11869, LARS 373, CPC 18946 | Medicago sativa | USA         | KM105223     |       |       |      |     |      |
| C. antinonicola          | CBS 102189*    | Antirrhinum majus | New Zealand | KM105180     |       |       |      |     |      |
| C. bryonicola            | CBS 109849*    | Bryonia dioica | Netherlands | KM105181     |       |       |      |     |      |
| C. destructivum          | CBS 114801, AR 4031 | Crupina vulgaris | Greece      | KM105219     |       |       |      |     |      |
|                          | CBS 119167, AR 4031 | Crupina vulgaris | Greece      | KM105220     |       |       |      |     |      |
|                          | CBS 128509, LARS 320 | Medicago sativa | Canada      | KM105214     |       |       |      |     |      |
|                          | CBS 157.83     | Medicago sativa | Serbia      | KM105215     |       |       |      |     |      |
|                          | CBS 511.97, LARS 202 | Medicago sativa | Morocco     | KM105216     |       |       |      |     |      |
|                          | CBS 520.97, LARS 709 | Medicago sativa | Saudi Arabia | KM105217     |       |       |      |     |      |
|                          | CBS 167.58     | Medicago sativa | Italy       | KM105213     |       |       |      |     |      |
|                          | CBS 130238, 5/11/1-1 | Phragmites | USA         | KM105218     |       |       |      |     |      |
|                          | IMI 387103, CPC 18082 | Rumex sp. | Korea       | KM105221     |       |       |      |     |      |
| C. fuscum                | CBS 136228*    | Trifolium hybridum | USA         | KM105207     |       |       |      |     |      |
|                          | CBS 136852     | Trifolium hybridum | USA         | KM105208     |       |       |      |     |      |
|                          | CBS 136853     | Trifolium hybridum | USA         | KM105209     |       |       |      |     |      |
|                          | CBS 136229     | Trifolium hybridum | USA         | KM105211     |       |       |      |     |      |
|                          | CBS 136230     | Trifolium repens | USA         | KM105210     |       |       |      |     |      |
|                          | CBS 136231     | Trifolium repens | USA         | KM105212     |       |       |      |     |      |
|                          | CBS 149.34     | Trifolium sp. | Netherlands | JQ005764     |       |       |      |     |      |
|                          | CBS 133704     | Digitalis dubia | Netherlands | KM105176     |       |       |      |     |      |
|                          | CBS 130.57     | Digitalis lanata | unknown | KM105174     |       |       |      |     |      |
|                          | CBS 133701*    | Digitalis lutea | Germany     | KM105174     |       |       |      |     |      |
|                          | CBS 133702     | Digitalis lutea | Netherlands | KM105178     |       |       |      |     |      |
|                          | CBS 133703     | Digitalis obscura | Netherlands | KM105175     |       |       |      |     |      |
|                          | CBS 85.68      | Digitalis purpurea | Netherlands | KM105177     |       |       |      |     |      |
|                          | CBS 200.54     | unknown       | Germany     | KM105179     |       |       |      |     |      |
| C. higginsianum          | Abc 6-2, CPC 19368 | Brassica chinensis | Japan      | KM105187     |       |       |      |     |      |
|                          | IMI 349061, CPC 19379* | Brassica chinensis | Trinidad and Tobago | KM105184     |       |       |      |     |      |
|                          | IMI 349063, CPC 19380 | Brassica chinensis | Trinidad and Tobago | JQ005760     |       |       |      |     |      |
|                          | Abo 1-1, CPC 19364 | Brassica oleracea | Gemmifera group | Japan | KM105185 |       |       |      |     |      |
|                          | Abp 1-2, CPC 19365 | Brassica pekinensis | Japan | KM105186 |       |       |      |     |      |
|                          | Abr 2-2, CPC 19369 | Brassica rapa | Japan | KM105188 |       |       |      |     |      |
|                          | Abr 3-1, CPC 19370 | Brassica rapa | Japan | KM105189 |       |       |      |     |      |
|                          | MAFF 305635, Abr 1-5, CPC 19366 | Brassica rapa | Pervivids Group | Japan | JQ005761 |       | JQ005803 | JQ005824 | JQ005845 |
|                          | CBS 128508, LARS 889, Kyoto 337-5 | Brassica rapa var. komatsuna | Japan | KM105190 |       |       |      |     |      |
|                          | NBRC 6182, CPC 18944 | Brassica sp. | Italy       | KM105191     |       |       |      |     |      |
|                          | AR 3-5, CPC 19363 | Raphanus sativus | Japan | KM105192 |       |       |      |     |      |
|                          | AR 3-1, CPC 19394 | Raphanus sativus | Japan | KM105193 |       |       |      |     |      |
|                          | AR 7-3, CPC 19395 | Raphanus sativus var. sativus | Japan | KM105194 |       |       |      |     |      |
|                          | AR 8-1, CPC 19396 | Raphanus sativus | Japan | KM105195 |       |       |      |     |      |

(continued on next page)
| Species         | Accession No. | Host                  | Country     | GenBank No.         |
|-----------------|---------------|-----------------------|-------------|---------------------|
|                 |               |                       |             | ITS                 |
| C. lentis       | CBS 127604, DAOM 235316, CT21* | *Lentilus culinaris* | Canada      | JQ005766            |
|                 | CBS 127605, DAOM 235317, CT26  | *Lentilus culinaris* | Canada      | KM105241            |
| C. linii        | CBS 172.51*   | *Linum usitatissimum* | Netherlands | JQ005765            |
|                 | CBS 505.97, LARS 77  | *Linum usitatissimum* | Ireland     | KM105226            |
|                 | IMI 103842, CPC 16847 | *Linum usitatissimum* | UK          | KM105227            |
|                 | IMI 103844, CPC 16816 | *Linum usitatissimum* | UK          | KM105228            |
|                 | CBS 112.21, LCP 46.621 | *Linum usitatissimum* | UK          | KM105229            |
|                 | CBS 100569, PD 97/14304 | *Nigella sp.*       | France      | KM105230            |
|                 | IMI 391904, IS320, CPC 19382 | *Raphanus raphanistrum* | Tunisia    | KM105232            |
|                 | CBS 117156    | *Teucrium scorodonia* | Netherlands | KM105231            |
|                 | CBS 136856    | *Medicago sativa*     | USA         | KM105233            |
|                 | CBS 136857    | *Taraxacum sp.*       | USA         | KM105239            |
|                 | CBS 136233    | *Taraxacum sp.*       | USA         | KM105240            |
|                 | CBS 136850    | *Trifolium hybridum*  | USA         | KM105237            |
|                 | CBS 136851    | *Trifolium hybridum*  | USA         | KM105238            |
|                 | CBS 130828    | *Trifolium repens*    | Germany     | KM105234            |
|                 | CBS 130829    | *Trifolium repens*    | Germany     | KM105235            |
|                 | IMI 69991, CPC 20242 | *Trifolium sp.*     | New Zealand | KM105236            |
| C. ocimi        | CBS 298.94*   | *Ocimum basilicum*    | Italy       | KM105222            |
| C. panacicola   | C08007        | *Panax ginseng*       | Korea       | GU935869            |
|                 | C08001        | *Panax ginseng*       | Korea       | GU935868            |
|                 | C08048        | *Panax ginseng*       | Korea       | GU935867            |
| C. pisicola     | CBS 724.97, LARS 60*  | *Plum satium*        | USA         | KM105172            |
| C. tabacum      | CBS 124249, MUCL 44942 | *Centella asiatica* | Madagascar | KM105206            |
|                 | N150, CPC 18945* | *Nicoticiana tabacum* | Canada      | KM105204            |
|                 | IMI 50187, CPC 16820 | *Nicoticiana tabacum* | India       | KM105205            |
|                 | CBS 161.53    | *Nicoticiana tabacum* | Zambia      | JQ005763            |
| C. tanacetii    | CBS 132693, BRIP 57314, UM01* | *Tanacetum cinerariifolium* | Australia | JX218228            |
|                 | CBS 132818, BRIP 57315, TAS000-0003 | *Tanacetum cinerariifolium* | Australia | JX218229            |
|                 | BRIP 57316, TAS000-0004 | *Tanacetum cinerariifolium* | Australia | JX218230            |
| C. ustrachense  | CBS 130243*   | *Trifolium pratense*  | Netherlands | KM105201            |
|                 | CBS 135827    | *Trifolium pratense*  | Netherlands | KM105202            |
|                 | CBS 135828    | *Trifolium pratense*  | Netherlands | KM105203            |
| C. vignae       | CBS 501.97, LARS 56*  | *Vigna unguiculata*  | Nigeria     | KM105183            |
|                 | IMI 334960, CPC 19383 | *Vigna unguiculata* | Nigeria     | KM105182            |
| Colletotrichum sp. | CBS 125336   | *Heracleum sp.*       | Netherlands | KM105198            |
|                 | CBS 126510    | *Heracleum sp.*       | Netherlands | KM105199            |
|                 | CPC 18076     | *Heracleum sp.*       | Netherlands | KM105200            |
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 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 overall topology of all of the equally most parsimonious trees was similar; they differed only in the position of isolates within the Colletotrichum 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 invariant 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 invariant 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 (PPs; see values plotted onto Fig. 1).

The results obtained 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. arthrininicola and C. byronicola and five unnamed strains. Colletotrichum utrecthense (78) and C. panacica (95/1.00) belonged to the second main clade, while the third clade only contained one subclade, representing C. tabacam (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. ochirin. 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 Psam 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,
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 19846 = 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 *Convolutus* 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 JX498034, 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. *Chlamydosporae* 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), polyphialidic 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–)4.5(–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
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 NZ, 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 on NCBI 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*.
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, (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*.

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.
Conidia of *C. bryonicola* 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 verrucose, 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 0.5–1.0 μm long, periclinal thickening visible. Conidia hyaline, smooth-walled, aseptate, cylindrical, straight to slightly curved, with both ends ± rounded, (14–)}
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 134.934 smaller, measuring (4.5–)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 134.934 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) × (3.5–)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, doliform to ampulliform, 5–14 × 2.5–5.5 μm, opening 1–2 μm diam, collettaire 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, fly 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 gum of Trifolium sp., 1 Oct. 1993, P.M.L. Tammes, culture CBS 149.34. USA, Utah, probably Salt Lake City, on stems and leaves of Trifolium pratense, 30 June, 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, MBB178515, 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 Garret Herbarium any more; the specimen was apparently sent away together with many specimens from that herbarium (M. Power, in litt.). 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. trifoli for 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 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 (GenBankKF800400, 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 (GenBankAY539866, Berner et al. 2004). Colletotrichum trifolii strain CBS 149.34 and C. cf. gloeosporioides strains 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, asseptate, 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,
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× (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, 5.5–7.5 μm diam, tip ± rounded to ± acute. Conidiophores hyaline to medium brown, smooth-walled, 3–160 μm long, 1–3-septate, base conical, 5.5–17.5 × 3–5.5 μm, opening 1–1.5 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, velvety 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 Emmensweg 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 136510; 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. antirrhinicolae). 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 002151 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 Ascocytha 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. unamuno/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. bryonica. 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. bryonica. Additionally, unnamed isolates from Heracleum are basal to C. fuscum, C. antirrhinicola, C. bryonica and C. vignae in our phylogeny, and could represent an additional, currently unidentifed 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), DAO18402 (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. Chlamydoospores not observed. Conidiomata co-nidiophores and setae on pale brown, angular cells, 3–9 μm. 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. Conidigenous 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, asep-tate, 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–)14.5–6.5(–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,
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 slightly curved, with one end rounded and the other truncate, (17.5–20(–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 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, on 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), 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 isoeptitype, 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 396582 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 (Schulzer 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. mathiolic, also resembles C. higginsianum and could be a synonym of this species or closely related based on similar conidia shape and size. Colletotrichum mathiolic 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. mathiolic.

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 (Djebari 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 Erca in the USA (GenBank KF550281, Patel et al. 2014), 12-223 (GenBank JX977428, K.S. Han et al., unpubl. data), C97027 and C99012 (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 (Gen-Bank 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
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 Battleford, 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, Iași, 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) epytified 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 the holotype 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 C10 and C11.

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 (Bhadaura et al. 2011) and functional analysis of a nudix hydrolase effector eliciting plant cell death (Bhadaura 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 charmoniale (Tripleurosperrum inodorum) in Canada (Forseille 2007). The potential of this fungus for biocontrol of scentless charmoniale was tested (Peng et al. 2005, Forseille et al. 2009). In the field, charmoniale 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 95SS5, 9971646, 95A8, 9970034 from lentil in Canada (GenBank AF451901, AF451904, AF451900, AF451903, Ford et al. 2004) and “Ga. glycines” isolate IF07384 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.) Tochihai, J. Coll. Agric. Hokkaido Imp. Univ. 14: 176. 1926. Fig. 9.
Basionym: Gloeosporium lini Westerd., Jaarversl. Phytopathol. Lab. “Willie Comminlin Scholten” 6. 1916 [1915].
= Colletotrichum liniola Petryhr. & 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
nidiophores and setae formed on pale brown, angular cells, 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 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(–6) × (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, asperate, 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, MBT177821; 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 194721; from seedling disease of Linum usitatissimum, collection date and collector unknown (deposited in CBS 194721).
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 van 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 150 × 4.2 ± 0.7 μ. These strains formed a subclade within the overall sequence variability within 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. cucodes (Liu et al. 2011). Wollenweber & Hochapfel (1949) also identified a collection from stems of Linum from Silesia as C. atramentarium.

Halhn (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. lini strains; the overall sequence variability within C. lini was higher. Moreover, their morphology was similar to strains from Nigella, Raphanus and Teucrum, 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 Teucrum 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 maiorum in steroid hydroxylations, to improve the biotransformation of steroids for the pharmaceutical industry, was studied by Romano et al. (2006).

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

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

**Sexual morph not observed.** Asexual morph on SNA. Vegetative hyphae 1–7 μm diam, hyaline, smooth-walled, septate, branched. Chlamydoospores 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. Conidigenous 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, 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. Conidigenous 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. Ganalbali, Inst. degli studi di Torino. Depart. di Valorizzazione e Protezione delle Risorse agroforestali) (CBS H-2164 holotype, culture ex-holotype CBS 216.94).

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. oicimi 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. oicimi 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. oicimi 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. aoteaora (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. oicimi is the same as that of C. destructivum (s. str.).

**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 as aseptate, cylindrical, straight or slightly curved conidia with rounded ends, measuring 17.0–22.1 × 3.4–5.1 μm, pyriform olive coloured appressoria,
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).

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
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 flaky 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, Feb. 1892, G. Lagerheim, BPI 399531, includes slide; Quito, from pods of Pisum sativum, Feb. 1892, G. Lagerheim, BPI 399532, includes slide; Quito, from pods of Pisum sativum, Feb. 1892, G. Lagerheim (No. 2944), BPI 399534.

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, LW 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, LW 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 longer 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 P. 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 P. 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 P. 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. falcatus 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 P. sativum 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 P. 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 P. sativum 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, claveate to navicular outline, with an entire or undulate margin, (4.5–)5.6–12(–15) × (3.5–)4.5–7.5(–9.5) μm, av. ± SD = 9.2 ± 2.7 × 5.9 ± 1.5 μm, LW 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, LW 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 P. 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 P. sativum in Russia as forming cylindrical-ovate, thick-walled conidia, measuring 10–18 × 5–6 μm that are mass pink and formed in

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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. karsii* (Damm et al. 2012). *Macrophoma sheldoni* was regarded as a synonym of *C. lagenarium* by Vassiljevski & Karakul (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. pisi* 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 a few 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 pales 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 doliform, 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 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*. MB178524, culture ex-neotype N150 = CPC 18945. *Germany*, Middle Franconia, from leaves of *Nicotiana rustica*, holotype, presumably lost. *India*, Rajahmundry, from *Nicotiana tabacum*, collection date unknown, B. S. Kadam, culture IMI 50187 = CPC 18920. *Madagascar*, from Centella asiatica, collection date and collector unknown (isolated by Rakotraina 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 M. Makul 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 microscopic 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 brownning 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).
Based on the description alone it is difficult to confirm whether *C. tabacum* and *Gm. nicotianae* are different species.

Lucas & Shew (1991) concluded *C. nicotianae* 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 species.

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**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.
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, setae, 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

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**Fig. 13. Colletotrichum tanaceti** (from ex-holotype strain CBS 132693). A–B. Conidiomata. C. Tip of a seta. D–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 cinerariofolium, Aug. 2010, S.J. Pethybridge, culture ex-holotype CBS 132693 = BRIP 57314 = UM01; Australia, northern Tasmania, Ulverstone, from Tanacetum cinerariofolium, collection date unknown, S.J. Pethybridge, living strain CBS 132818 = BRIP 57315 = TAS060-0003.

Notes: Pyrethrum (Tanacetum cinerariofolium, 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.
**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, pericllinal 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, ellipsoid 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 μm, L/W ratio = 1.8.

**Asexual morph on Anthriscus stem. Conidiomata** absent, conidiophores and setae formed directly on hyphae, or rarely on pale brown, angular cells, 3.5–6 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, cylindrical to conical, 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 white to yellowish 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 135826.

**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 (Gen-Bank 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. fuscus* 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, polyphialidic 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, sub-globose 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 = 2.8.
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

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**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.
von Arx (1957, wrongly cited as C. phasorum). 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 growing species within the Barimani et al. experiments in the laboratory (Armstrong-Cho & Banniza 2006, erothallic and sexual morphs were only observed by crossing

cies studied in the 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. spathelianum 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. pisciola, C. tanaceti), while others are almost straight (especially C. tabacum and C. oicim) and reminiscent of C. cocodes 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. cocodes, C. truncatum, C. gloeosporioides, C. lindemuthianum or C. trifoli. 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. lenti (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. pisciola, 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. lenti) 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 (Auoyng 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* var. 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. *pekinesis*), 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. vigneae in this study (see notes under C. vigneae). 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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