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Diversity of *Colletotrichum* species on strawberry (*Fragaria* × *ananassa*) in Germany

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Summary. Anthracnose caused by *Colletotrichum* species is an important disease of strawberries (*Fragaria* × *ananassa*), but the species causing this disease in Germany have not been investigated based on modern systematics. By using multi-locus phylogenetic analyses (ITS, *act*, *gapdh*, *chs-1*, *his3*, *tub2*), 58 *Colletotrichum* isolates from previous and recent collections, obtained mainly from fruit anthracnose of cultivated strawberries in Germany, were identified or re-identified as *C. fioriniae*, *C. godetiae* and *C. nymphaeae* (*C. acutatum* species complex) as well as *C. anthrisci* and *C. lineola* (*C. dematium* complex). *Colletotrichum nymphaeae* was dominant; most of the isolates belonged to one clonal lineage that occurs on strawberries throughout Europe, the United States of America, and some African and Asian countries. One of the other two haplotypes was distantly related and only represented by recently collected material. All other species, each of one haplotype, had only been isolated once or twice from German strawberries. This is the first report of *C. anthrisci* in Germany and for the genus *Fragaria* worldwide; all the other isolated fungi are newly reported for this genus in Germany. Comparisons of morphological characteristics of the species identified demonstrate that these features are of limited use for identification, even to species complex level. In pathogenicity tests, all five species caused anthracnose symptoms on ripe fruit of *Fragaria* × *ananassa* 'Asia'.

Keywords. Anthracnose, *Colletotrichum acutatum*, *C. dematium*, multi-locus phylogeny, pathogenicity.

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INTRODUCTION

Strawberries (*Fragaria* × *ananassa*, *Rosaceae*) are the most common fruit crops after apples in Germany, with a harvested volume of 133,135 t in 2022 (Lehari, 2002; Statistisches Bundesamt, 2023). Intensive strawberry breeding, which has been primarily aimed at high yields, influenced the gene structure and thus immunity to pathogens in strawberries (Hardigan *et al*., 2018), so strawberry cultivation is associated with high production risks. In the presence of warm temperatures and high humidity, rapid outbreaks of anthracnose caused by *Colletotrichum* species can be expected (Howard *et al*., 1992). Trading of young plantlets with latent infections can rapidly spread the disease over long distances (Howard *et al*., 1992). Anthracnose of strawberry

fruit is characterised as brown, sunken spots, in which orange conidial mucilage develops. Main symptoms of green tissues (runners, leaves and stalks) include dark, sunken, necrotic areas (Howard *et al*., 1992).

Brooks (1931) reported a new strawberry disease in Florida, United States of America (USA), called anthracnose, and described the causative pathogen as *C. fragariae*. In the 1980s, *C. acutatum sensu lato* was first reported from strawberries in the USA (Smith and Black, 1986). Anthracnose symptoms in strawberry fields in Germany (Baden, Palatinate) and other European countries including France, Bulgaria, Sweden, Denmark, Spain and Belgium have been reported at least since the 1990s and attributed to *C. acutatum* and *C. gloeosporioides*, while *C. fragariae* was only found within material from the USA (Denoyes and Baudry, 1995; Laun and Fried, 1996; Bobev *et al*., 2002; Nilsson *et al*., 2005; Sundelin *et al*., 2005; Garrido *et al*., 2008; Debode *et al*., 2009). The first molecular study of *Colletotrichum* in Germany by Nirenberg *et al.* (2002) focusing on *C. lupini*, included a strain from strawberry in Germany that had also been identified as *C. acutatum* based on ITS sequence data. Within this investigation, several *Colletotrichum* strains from different hosts and countries, including from strawberries in Germany, had been collected and are maintained at the culture collection of the Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) Berlin, now Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen (Federal Research Centre for Cultivated Plants), Institut für Epidemiologie und Pathogendiagnostik, Braunschweig, Germany.

The systematics of *Colletotrichum* has changed within the last 20 years, due to application of molecular data, especially in multi-locus DNA sequence analyses. *Colletotrichum acutatum* and *C. gloeosporioides* that had been previously regarded as causal agents of strawberry anthracnose were shown to be large species complexes (Damm *et al*., 2012a; Weir *et al*., 2012). The number of accepted *Colletotrichum* species, those that have been revised or newly described based on multi-locus DNA sequences, is constantly increasing; more than 300 *Colletotrichum* species in at least 15 species complexes and more than ten further species are currently accepted (Liu *et al*., 2022; Talhinhas and Baroncelli, 2023). To date, at least 22 species are known from strawberry, belonging to the *C. acutatum*, *C. boninense*, *C. coccodes*, *C. dematium*, *C. gloeosporioides* and *C. truncatum* species complexes (Farr and Rossman, 2024). *Colletotrichum acutatum sensu stricto* is only confirmed from strawberry in Australia (Damm *et al*., 2012a), while most reports of *C. acutatum* on strawberry in the USDA Fungal Databases (Farr and Rossman, 2024), including that from Germany (Nirenberg *et al*., 2002), date back to the pre-molecular era, or were from before *C. acutatum* was shown to be a species complex (Damm *et al*., 2012a). Therefore, these fungi should be considered as *C. acutatum sensu lato* and could include other species within this complex.

To date, only *Colletotrichum* strains infecting a few random, often exotic host plants collected in Germany have been identified or described based on modern systematics (e.g. Damm *et al*., 2012a, b, 2014, 2019; Weir *et al*., 2012); while there are no records of *Colletotrichum* species from *Fragaria* hosts in Germany identified on the same basis.

The aims of the present study were to investigate the diversity of *Colletotrichum* on strawberries from all over Germany based on multi-locus sequence data, to characterise the species morphologically and to test their pathogenicity to fruit of cultivated strawberry (*Fragaria* × *ananassa*) 'Asia'.

MATERIALS AND METHODS

Isolates

Symptomatic material of cultivated strawberry plants (*Fragaria* × *ananassa*) was collected in different regions of Germany, including Saxony (Dresden, Görlitz, Ore Mountains), Brandenburg (Spreewald), North Rhine-Westphalia (Münsterland), Lower Saxony (Lüdersfeld) and Mecklenburg-Western Pomerania (Mecklenburg Lake District). The collected material also included fruit bought at markets in Germany (some of Polish origin) and one sample from wild strawberry (*Fragaria vesca*).

Most of the newly collected material consisted of infected fruit of *F.* × *ananassa*, which had symptoms of brown, sunken spots of necrotic tissue that spread radially. Orange conidial masses sometimes developed in the centres of these spots. Flat, pale to dark mouse-grey mycelium often formed at the edges and sometimes over the entire infection sites. Elongated dark brown necroses initially formed on host stems and petioles, which spread and developed into stem-encompassing necroses and constrictions, on which conidia were sometimes observed. On affected leaves of *F.* × *ananassa*, roundish to oval necrotic areas were observed, each with dark brown irregular edges and a light brown centre, which partially merged. Symptoms of the *F. vesca* leaf sample were similar; grey-brown spots of various sizes and shapes with paler centres and darker irregular margins formed especially at the edges of affected leaves.

To obtain single-conidium isolates, conidia formed on the necrotic host tissues were spread on the surface

of petri dishes with synthetic nutrient-poor agar (SNA; Nirenberg, 1976) using a drop of sterile water. On the following day, single germinating conidia were transferred to oatmeal agar (OA; Crous *et al*., 2019). Plant parts, on which no conidia were present, were surface sterilised (1 min in 3.5% NaClO, 30 s in 70% ethanol), washed in sterile water and placed in petri dishes on sterile filter paper with sterile water until conidia were formed, from which single-conidium isolates were produced.

A further 22 isolates from the culture collection of the former BBA had been collected more than 20 years ago in the federal states of Mecklenburg-Western Pomerania, Saxony, Baden-Württemberg, Hesse, Rhineland-Palatinate and Brandenburg and included one strain bought in a supermarket that originated from the Netherlands.

Although it was not possible to complete and confirm all collection data, it is assumed that all the BBA isolates originated from anthracnose symptoms, especially from fruit rot, because the material had been deposited by plant protection offices in different German federal states in the years when strawberry anthracnose spread across Europe. For one of the isolates from Saxony there was information provided that a 2-year-old stock of 'Selva' (imported plants) was very heavily infested, and a 1-year-old stock was over 50% infected. Most of the strawberry runners from which isolates had been obtained were characterised as necrotic, while one isolate had a noticeable reddish discolouration at the infection site. Two isolates originated from brown roots of *F. vesca* var. *semperflorens*. There was no information on the leaf symptom and no further information on fruit symptoms than fruit rot.

The isolates were stored in the culture collection of the Senckenberg Museum of Natural History Görlitz, Germany (GLMC; Table 1). Selected isolates were deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany.

Morphological analyses

The *Colletotrichum* strains were cultivated on SNA with autoclaved filter paper and double-autoclaved stems of *Anthriscus sylvestris* and on OA. Cultures were incubated at 20°C under near UV light (12 h daily photoperiod) for 10 d. Microscopic preparations were made in clear lactic acid. Measurements of microscopic structures of the fungi were carried out according to Damm *et al*. (2007), with 30 measurements per structure and strain, using a Nikon SMZ1000 dissecting microscope (DM) and a Nikon Eclipse 80i compound microscope with differential interference contrast (DIC). Appressoria were observed on the reverse sides of the SNA cultures. Colony features on SNA and OA were observed after the incubation period. To calculate colony growth rates, the diameters of the colonies were determined after 7 and 10 d. Colony colour was characterised according to Rayner (1970).

Phylogenetic analyses

Genomic DNA was extracted from cultures according to Damm *et al*. (2008). The 5.8S nuclear ribosomal RNA gene with the two flanking internal transcribed spacers (ITS), a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (*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 respective 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 and Kohn, 1999), CYLH3F + CYLH3R (Crous *et al*., 2004), ACT-512F + ACT-783R (Carbone and Kohn, 1999) and T1 (O'Donnell and Cigelnik, 1997) + Bt-2b (Glass and Donaldson, 1995) or T1 + BT4R (Woudenberg *et al*., 2009). DNA amplifications were carried out in a Mastercycler® pro S (Eppendorf), each in a total volume of 20 µL. The PCR reaction mixture contained 1 µL of 1:10 diluted genomic DNA, 2.5 µL of 10× buffer (Peqlab), 1 µL of each primer (10 mM), 2.5 µL of $MgCl_2$ (25 mM), 2.5 µL of dNTPs (2 mM), 0.7 µL of DMSO and 0.1 µL of *Taq* DNA polymerase (0.5 U; Peqlab). Conditions for PCR of *gapdh*, *chs-1*, *his3*, *act*, and *tub2* included an initial denaturation step of 5 min at 94°C, followed by 40 cycles each 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. ITS PCR was carried out as described by Woudenberg *et al*. (2009). PCR products were visualised on a 1% agarose gel and sequenced by the Senckenberg Biodiversity and Climate Research Centre (BiK-F) laboratory (Frankfurt, Germany). The DNA sequences generated with forward and reverse primers were used to obtain consensus sequences, using BioNumerics v. 7.6.3 (Applied Maths, St-Marthens-Lathem, Belgium), and the alignments were assembled and adjusted manually using Sequence Alignment Editor v. 2.0a11 (Rambaut, 2002). Sequences derived in this study were lodged at NCBI GenBank (www.ncbi.nlm.nih.gov).

For preliminary identification and selection of reference strains, blastn searches were carried out with ITS sequences in NCBI GenBank. Maximum parsimony (MP) analyses were carried out on the multi-locus alignments (ITS, *gapdh*, *chs-1*, *his3*, *act*, *tub2*) with Phy-

logenetic Analysis Using Parsimony (PAUP) v. 4.0b10 (Swofford, 2003), using the heuristic search option with 100 random sequence additions and tree bisection and reconstruction as the branch-swapping algorithm. Alignment gaps were treated as missing, and all characters were unordered and of equal weight. No more than ten trees of score (length) greater than or equal to ten were saved in each replicate. Tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for the resulting trees. The robustness of the obtained trees was evaluated by 10,000 bootstrap replications, using the fast-stepwise addition algorithm (Hillis and Bull, 1993). Maximum likelihood (ML) analyses were calculated online using IQ-TREE 1.6 (http://iqtree.cibiv.univie.ac.at; Nguyen *et al*., 2015; Trifinopoulos *et al*., 2016) and model testing under the Bayesian information criterion (BIC) (Kalyaanamoorthy *et al*., 2017). Branch support was obtained by 5000 replicates of ultrafast bootstrap (ufb; Hoang *et al*., 2018; Minh *et al*., 2013). Support values ≥ 95% were considered relevant, following Guindon *et al.* (2010).

Pathogenicity tests

Selected *Colletotrichum* isolates were incubated on OA at 20°C under near UV light for 10 d. To harvest the conidia, 10 mL of sterile distilled water was added to an OA culture of each isolate and swirled thoroughly. The resulting conidium suspensions were adjusted to a final concentration of 2×10^4 conidia mL^{-1}. Ripe fruit of *F.* × *ananassa* 'Asia' (Obsthof Rüdiger, Dresden, Germany) of the quality standard EU No. 543/2011 class Extra (Anonymous, 2011) were surface sterilised with 1% NaClO for 6 min, washed three times with sterile distilled water and placed under sterile conditions on moist filter paper in covered glasses (Gourmet glass, round edge, 300 mL, Weck). Each fruit was inoculated with a 5 µL droplet of conidium suspension (without wounding) or treated with 5 µL of sterile distilled water (experimental control). Five repetitions (fruit) were made for each *Colletotrichum* strain and the control. The covered glasses with inoculated strawberries were placed randomly in a climate cabinet (20°C, 14 h fluorescent light/10 h dark daily cycle, humidity up to 100%). The experiment was carried out twice. Seven d post inoculation (dpi), resulting lesion sizes were determined by counting infected unit squares on a grid placed over scaled photographs. Symptoms were evaluated visually and photographed 7 dpi or, in the case of delayed infection development, 10 dpi. Re-isolations were made from lesion edges and resulting fungi were identified.

RESULTS

Phylogenetic analyses

Based on preliminary morphological examinations and blastn searches of the ITS sequences on NCBI GenBank, 56 of the 58 isolates were identified as belonging to the *C. acutatum* species complex. The other two isolates were identified as belonging to the *C. dematium* complex.

In the multi-locus phylogenetic analyses of the *C. acutatum* species complex (gene boundaries of ITS: 1–546, *gapdh*: 557–829, *chs-1*: 840–1121, *his3*: 1132–1519, *act*: 1530–1777, *tub2*: 1788–2285) 181 isolates, including a selection of 32 (of the 56) newly sequenced isolates from strawberry, 147 reference strains of the *C. acutatum* species complex, and the outgroup *C. orchidophilum* CBS 632.80 and IMI 309357 (Table 1, Supplementary table 1) and 2285 characters including the alignment gaps were processed. Of these, 429 characters were parsimony-informative, 154 parsimony-uninformative and 1702 constant. After a heuristic search using PAUP, the maximum of 590 equally most parsimonious trees were retained (length = 1203 steps, CI = 0.615, RI = 0.947, RC = 0.582, HI = 0.385). One of these trees is shown in Figure 1. The topology of these trees was similar, which was verified for a large section of trees. They differed in the position of taxa within subclades. The consensus tree of the ML analysis confirmed the tree topology obtained with parsimony. The bootstrap values of the two analyses generally agreed with each other. The phylogeny consists of five main clades representing clades 1 to 5 in Damm *et al.* (2012a). Most of the isolates clustered within *C. nymphaeae* (clade 2), where they formed three haplotype groups; most of the strains were of one haplotype. This was the same as several other isolates from strawberry in Europe, Northern America and Africa. According to a preliminary analysis, 46 isolates from the present study belonged to this haplotype, and 24 of these isolates were included in the final analyses. Isolates GLMC 2599, GLMC 2600 and GLMC 2606 represented a second haplotype with other strains from strawberry in Europe and the USA and a strain from *Anemone* in the Netherlands. The third haplotype included isolates GLMC 2653, GLMC 2654 and a strain from *Fragaria* in the USA, forming a distinct subclade (bootstrap support MP/ML: -/100). Two isolates (GLMC 2660, GLMC 2661) clustered in *C. fioriniae* (clade 3); the haplotype was the same as that of a strain from apple in Italy and from *Grevillea* in Germany. Three further strains, two from Germany (GLMC 2589, GLMC 2590) and one from the Netherlands (GLMC 2583), grouped with *C. godetiae* (clade 5). This haplotype was the same as that of strains

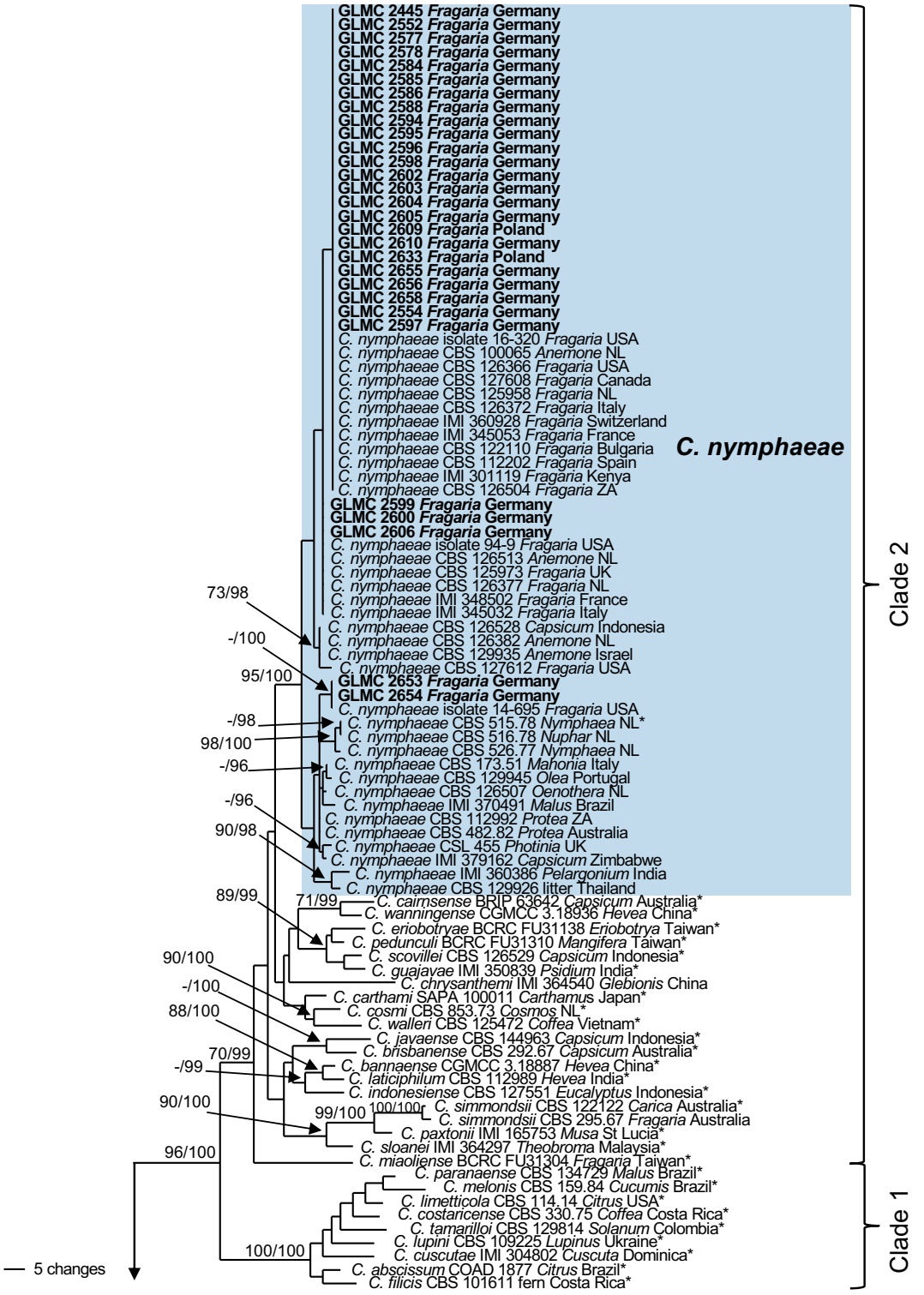

Figure 1. The first of 590 most parsimonious trees obtained from a heuristic search of the combined ITS, *act*, *gapdh*, *chs-1*, *his3*, *tub2* sequence alignment of *Colletotrichum* isolates from *Fragaria* and representative strains of the *C. acutatum* species complex. Bootstrap support values of the MP analysis >70% and of the ML analysis >95% are shown at the nodes. *Colletotrichum orchidophilum* CBS 632.80 and IMI 309357 were used as outgroup. Numbers of ex-type strains are indicated by an asterisk. Isolates obtained or sequenced in this study are shown in bold font. Strain numbers are followed by substrate (host genus) and country of origin (NL = Netherlands, NZ = New Zealand, ZA = South Africa). (Continued)

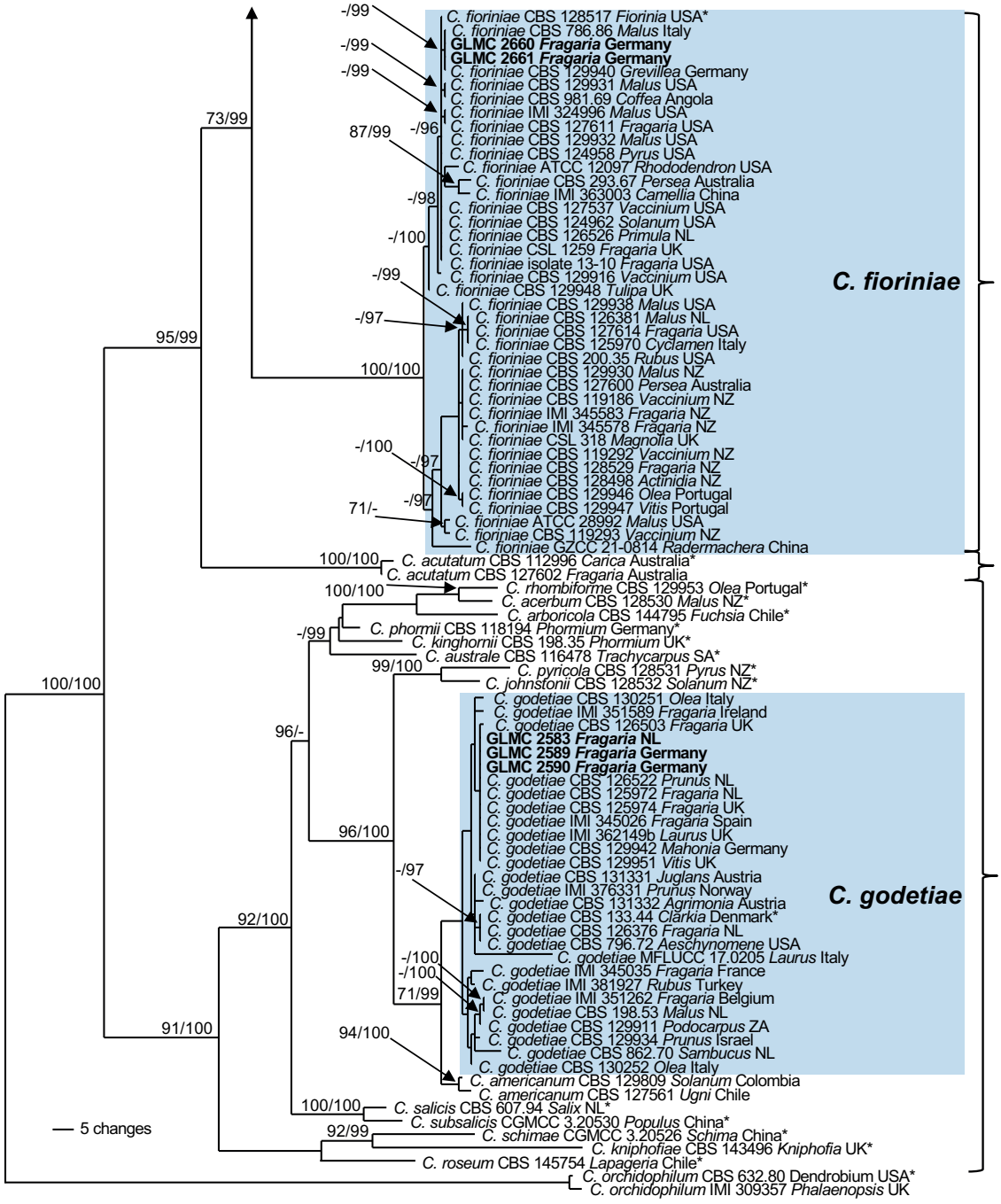

Figure 1. (Continued).

from strawberry and other hosts from other European countries, including an isolate from *Mahonia* in Germany.

In the multi-locus phylogenetic analyses of the *C. dematium* species complex (gene boundaries of ITS: 1–530, *gapdh*: 541–821, *chs-1*: 832–1082, *his3*: 1093–1489, *act*: 1500–1768, *tub2*: 1779–2298), 36 isolates, including the two newly sequenced isolates from strawberry, 33 reference strains and the outgroup *C. chlorophyti* IMI 103806 (Table 1, Supplementary table 1), along with 2298 characters including the alignment gaps were processed. Of these, 415 characters were parsimony-informative, 205 parsimony-uninformative and 1678 constant. After a heuristic search using PAUP, the maximum of 440 equal-

Table 1. Strains of *Colletotrichum* spp. used in this study, with collection details and GenBank accession numbers.

Species	Accession No.[a]	Host/Substrate	Country and Federal State of Germany	GenBank No.[b]					
				ITS	*gapdh*	*chs-1*	*his3*	*act*	*tub2*
C. anthrisci	GLMC 2616, DSM 115225	*Fragaria vesca*, leaf spots	Germany, Saxony	PP069452	PP115710	PP115876	PP115916	PP115767	PP115825
C. fioriniae	GLMC 2660, DSM 115228	*Fragaria* × *ananassa*, fruit rot	Germany, Saxony	PP069446	PP115704	PP115870	—	PP115761	PP115819
C. fioriniae	GLMC 2661, DSM 115229	*Fragaria* × *ananassa*, fruit rot	Germany, Saxony	PP069447	PP115705	PP115871	PP115911	PP115762	PP115820
C. godetiae	GLMC 2589, BBA 70063, DSM 115222	*Fragaria* × *ananassa*, runner with necroses	Germany, Baden-Württemberg	PP069449	PP115707	PP115873	PP115913	PP115764	PP115822
C. godetiae	GLMC 2590, BBA 71234, DSM 115223	*Fragaria* × *ananassa*, fruit	Germany, Mecklenburg-Western Pomerania	PP069450	PP115708	PP115874	PP115914	PP115765	PP115823
C. godetiae	GLMC 2583, BBA 68320	*Fragaria* × *ananassa*, fruit	Netherlands, bought at Plus Markt Berlin, Germany	PP069448	PP115706	PP115872	PP115912	PP115763	PP115821
C. lineola	GLMC 2587, BBA 71830, DSM 115221	*Fragaria* × *ananassa*	Germany, Brandenburg	PP069451	PP115709	PP115875	PP115915	PP115766	PP115824
C. nymphaeae	GLMC 2597, BBA 70822	*Fragaria* × *ananassa*, fruit	Germany, Hesse	PP069424	PP115682	PP115848	—	PP115739	PP115797
C. nymphaeae	GLMC 2554	*Fragaria* × *ananassa*, 'Jive', fruit rot	Germany, Lower Saxony	PP069409	PP115667	PP115833	—	PP115725	PP115782
C. nymphaeae	GLMC 2605, BBA 70094	*Fragaria* × *ananassa*	Germany, Saxony	PP069431	PP115689	PP115855	—	PP115746	PP115804
C. nymphaeae	GLMC 2585, BBA 67865	*Fragaria* × *ananassa*, runner with necroses	Germany, Baden-Württemberg	PP069418	PP115676	PP115842	—	PP115734	PP115791
C. nymphaeae	GLMC 2602, BBA 67859	*Fragaria* × *ananassa*, leaf	Germany, Baden-Württemberg	PP069428	PP115686	PP115852	PP115896	PP115743	PP115801
C. nymphaeae	GLMC 2603, BBA 67866	*Fragaria* × *ananassa*, runner with necroses	Germany, Baden-Württemberg	PP069429	PP115687	PP115853	PP115897	PP115744	PP115802
C. nymphaeae	GLMC 2595, BBA 68394	*Fragaria* × *ananassa*, fruit	Germany, Baden-Württemberg?	PP069422	PP115680	PP115846	PP115891	—	PP115795
C. nymphaeae	GLMC 2596, BBA 70766	*Fragaria* × *ananassa*	Germany, Brandenburg	PP069423	PP115681	PP115847	PP115892	PP115738	PP115796
C. nymphaeae	GLMC 2653, DSM 115226	*Fragaria* × *ananassa*, fruit rot	Germany, Brandenburg	PP069439	PP115697	PP115863	PP115904	PP115754	PP115812
C. nymphaeae	GLMC 2654	*Fragaria* × *ananassa*, fruit rot	Germany, Brandenburg	PP069440	PP115698	PP115864	PP115905	PP115755	PP115813
C. nymphaeae	GLMC 2655	*Fragaria* × *ananassa*, fruit rot	Germany, Brandenburg	PP069441	PP115699	PP115865	PP115906	PP115756	PP115814
C. nymphaeae	GLMC 2598, BBA 70821	*Fragaria* × *ananassa*, fruit	Germany, Hesse	PP069425	PP115683	PP115849	PP115893	PP115740	PP115798
C. nymphaeae	GLMC 2599, BBA 72544	*Fragaria vesca* var. *semperflorens*, brown roots	Germany, Hesse	PP069426	PP115684	PP115850	PP115894	PP115741	PP115799
C. nymphaeae	GLMC 2600, BBA 72543, DSM 115224	*Fragaria vesca* var. *semperflorens*, brown roots	Germany, Hesse	PP069427	PP115685	PP115851	PP115895	PP115742	PP115800
C. nymphaeae	GLMC 2604, BBA 72341	*Fragaria* × *ananassa*, runner with reddish discoloration	Germany, Hesse	PP069430	PP115688	PP115854	PP115898	PP115745	PP115803
C. nymphaeae	GLMC 2552	*Fragaria* × *ananassa*, 'Jive', fruit rot	Germany, Lower Saxony	PP069407	PP115665	PP115831	PP115881	PP115723	PP115780
C. nymphaeae	GLMC 2553	*Fragaria* × *ananassa*, 'Jive', fruit rot	Germany, Lower Saxony	PP069408	PP115666	PP115832	PP115882	PP115724	PP115781

(Continued)

Table 1. (Continued).

Species	Accession No.[a]	Host/Substrate	Country and Federal State of Germany	GenBank No.[b]					
				ITS	*gapdh*	*chs-1*	*his3*	*act*	*tub2*
C. nymphaeae	GLMC 2555	*Fragaria* × *ananassa*, 'Jive', fruit rot	Germany, Lower Saxony	PP069410	PP115668	PP115834	PP115883	PP115726	PP115783
C. nymphaeae	GLMC 2556	*Fragaria* × *ananassa*, 'Jive', fruit rot	Germany, Lower Saxony	PP069411	PP115669	PP115835	PP115884	PP115727	PP115784
C. nymphaeae	GLMC 2557	*Fragaria* × *ananassa*, 'Jive', fruit rot	Germany, Lower Saxony	PP069412	PP115670	PP115836	PP115885	PP115728	PP115785
C. nymphaeae	GLMC 2558	*Fragaria* × *ananassa*, 'Jive', fruit rot	Germany, Lower Saxony	PP069413	PP115671	PP115837	PP115886	PP115729	PP115786
C. nymphaeae	GLMC 2559	*Fragaria* × *ananassa*, 'Jive', fruit rot	Germany, Lower Saxony	PP069414	PP115672	PP115838	PP115887	PP115730	PP115787
C. nymphaeae	GLMC 2610	*Fragaria* × *ananassa*, fruit rot	Germany, Mecklenburg-Western Pomerania	PP069434	PP115692	PP115858	PP115901	PP115749	PP115807
C. nymphaeae	GLMC 2611	*Fragaria* × *ananassa*, fruit rot	Germany, Mecklenburg-Western Pomerania	PP069435	PP115693	PP115859	—	PP115750	PP115808
C. nymphaeae	GLMC 2612	*Fragaria* × *ananassa*, fruit rot	Germany, Mecklenburg-Western Pomerania	PP069436	PP115694	PP115860	PP115902	PP115751	PP115809
C. nymphaeae	GLMC 2656, DSM 115227	*Fragaria* × *ananassa*, 'Asia', petiole with brown spots	Germany, North Rhine-Westphalia	PP069442	PP115700	PP115866	PP115907	PP115757	PP115815
C. nymphaeae	GLMC 2657	*Fragaria* × *ananassa*, 'Asia', petiole with brown spots	Germany, North Rhine-Westphalia	PP069443	PP115701	PP115867	PP115908	PP115758	PP115816
C. nymphaeae	GLMC 2658	*Fragaria* × *ananassa*, 'Asia', leaf spots	Germany, North Rhine-Westphalia	PP069444	PP115702	PP115868	PP115909	PP115759	PP115817
C. nymphaeae	GLMC 2659	*Fragaria* × *ananassa*, 'Asia', leaf spots	Germany, North Rhine-Westphalia	PP069445	PP115703	PP115869	PP115910	PP115760	PP115818
C. nymphaeae	GLMC 2588, BBA 68332	*Fragaria* × *ananassa*, fruit	Germany, Rhineland-Palatinate?	PP069420	PP115678	PP115844	PP115890	PP115736	PP115793
C. nymphaeae	GLMC 2594, BBA 68333	*Fragaria* × *ananassa*, fruit	Germany, Rhineland-Palatinate?	PP069421	PP115679	PP115845	—	PP115737	PP115794
C. nymphaeae	GLMC 2445, DSM 115220	*Fragaria* × *ananassa*, 'Asia', fruit rot	Germany, Saxony	PP069396	PP115654	PP115827	PP115877	PP115712	PP115769
C. nymphaeae	GLMC 2446	*Fragaria* × *ananassa*, 'Asia', fruit rot	Germany, Saxony	PP069397	PP115655	—	—	PP115713	PP115770
C. nymphaeae	GLMC 2447	*Fragaria* × *ananassa*, 'Faith', fruit rot	Germany, Saxony	PP069398	PP115656	—	—	PP115714	PP115771
C. nymphaeae	GLMC 2448	*Fragaria* × *ananassa*, 'Faith', fruit rot	Germany, Saxony	PP069399	PP115657	PP115828	PP115878	PP115715	PP115772
C. nymphaeae	GLMC 2449	*Fragaria* × *ananassa*, 'Diana', fruit rot	Germany, Saxony	PP069400	PP115658	PP115829	PP115879	PP115716	PP115773
C. nymphaeae	GLMC 2450	*Fragaria* × *ananassa*, fruit rot	Germany, Saxony	PP069401	PP115659	—	—	PP115717	PP115774

(Continued)

Table 1. (Continued).

Species	Accession No.[a]	Host/Substrate	Country and Federal State of Germany	GenBank No.[b]					
				ITS	*gapdh*	*chs-1*	*his3*	*act*	*tub2*
C. nymphaeae	GLMC 2451	*Fragaria* × *ananassa*, fruit rot	Germany, Saxony	PP069402	PP115660	—	—	PP115718	PP115775
C. nymphaeae	GLMC 2452	*Fragaria* × *ananassa*, fruit rot	Germany, Saxony	PP069403	PP115661	—	—	PP115719	PP115776
C. nymphaeae	GLMC 2453	*Fragaria* × *ananassa*, fruit rot	Germany, Saxony	PP069404	PP115662	—	—	PP115720	PP115777
C. nymphaeae	GLMC 2454	*Fragaria* × *ananassa*, stem with brown spots	Germany, Saxony	PP069405	PP115663	—	—	PP115721	PP115778
C. nymphaeae	GLMC 2455	*Fragaria* × *ananassa*, stem with brown spots	Germany, Saxony	PP069406	PP115664	PP115830	PP115880	PP115722	PP115779
C. nymphaeae	GLMC 2577, BBA 70090	*Fragaria* × *ananassa*	Germany, Saxony	PP069415	PP115673	PP115839	—	PP115731	PP115788
C. nymphaeae	GLMC 2584, BBA 70095	*Fragaria* × *ananassa*	Germany, Saxony	PP069417	PP115675	PP115841	—	PP115733	PP115790
C. nymphaeae	GLMC 2586, BBA 70092	*Fragaria* × *ananassa*	Germany, Saxony	PP069419	PP115677	PP115843	PP115889	PP115735	PP115792
C. nymphaeae	GLMC 2606, BBA 70093	*Fragaria* × *ananassa*	Germany, Saxony	PP069432	PP115690	PP115856	PP115899	PP115747	PP115805
C. nymphaeae	GLMC 2578, BBA 70091	*Fragaria* × *ananassa*, 'Selva', fruit rot	Germany, Saxony, young plants imported from France via Netherlands	PP069416	PP115674	PP115840	PP115888	PP115732	PP115789
C. nymphaeae	GLMC 1819, 494-99	*Fragaria* × *ananassa*	Germany?, Brandenburg?	PP069395	PP115653	PP115826	—	PP115711	PP115768
C. nymphaeae	GLMC 2609	*Fragaria* × *ananassa*, fruit rot	Poland, bought at the market in Görlitz, Germany	PP069433	PP115691	PP115857	PP115900	PP115748	PP115806
C. nymphaeae	GLMC 2633	*Fragaria* × *ananassa*, fruit rot	Poland, bought at the market in Görlitz, Germany	PP069437	PP115695	PP115861	PP115903	PP115752	PP115810
C. nymphaeae	GLMC 2634	*Fragaria* × *ananassa*, fruit rot	Poland, bought at the market in Görlitz, Germany	PP069438	PP115696	PP115862	—	PP115753	PP115811

[a] GLMC: Culture collection of Senckenberg Museum of Natural History Görlitz, Görlitz, Germany; BBA: Culture collection of the Biologische Bundesanstalt für Land und Forstwirtschaft, Berlin, Germany; DSM: German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. [b] ITS, 5.8S nuclear ribosomal RNA gene with the two flanking internal transcribed spacers; *gapdh*, 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase gene; *chs-1*, partial sequences of the chitin synthase 1 gene; *his3*, partial sequences of the histone H3 gene; *act*, partial sequences of the actin gene; *tub2*, partial sequences of the β-tubulin gene.

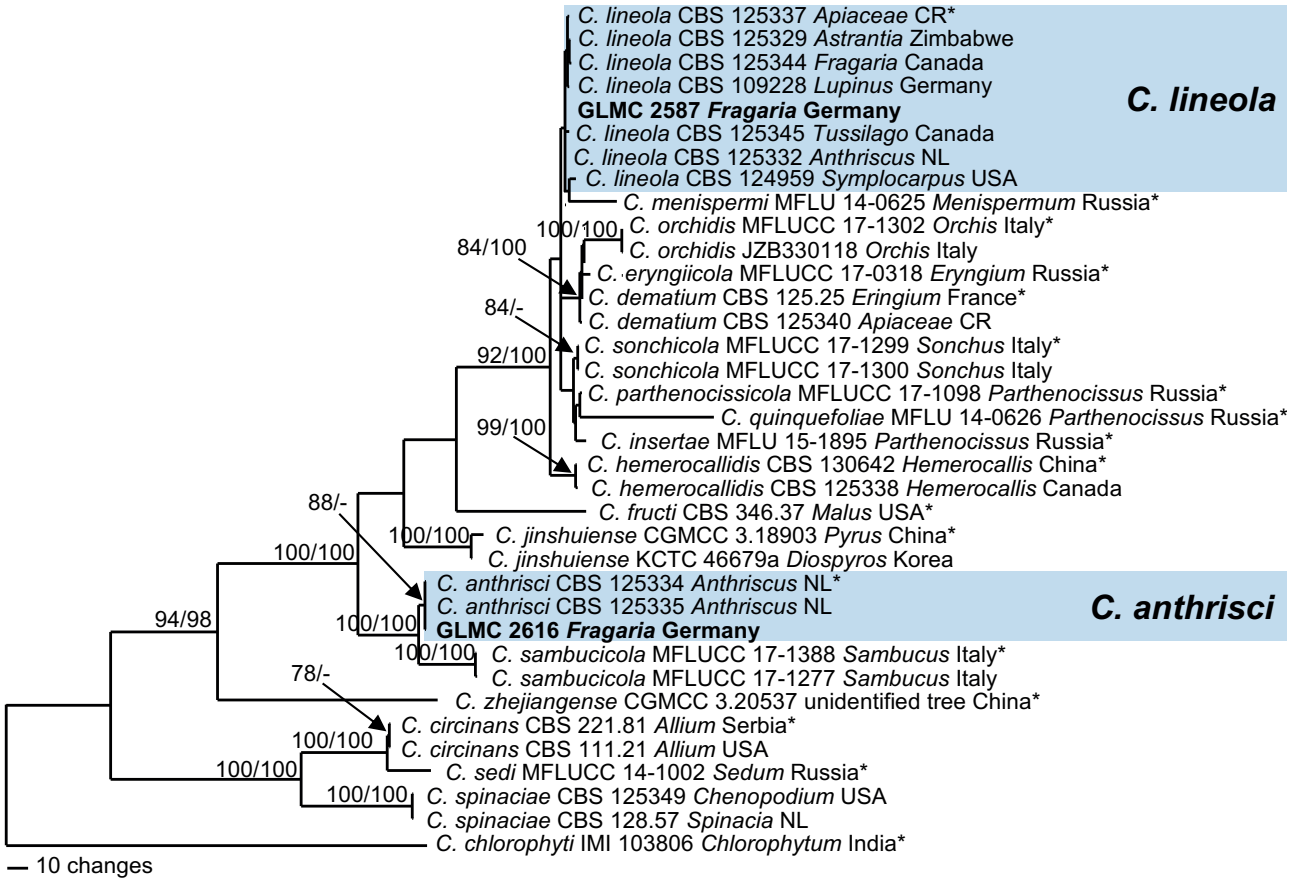

Figure 2. The first of 440 most parsimonious trees obtained from a heuristic search of the combined ITS, *act*, *gapdh*, *chs-1*, *his3*, *tub2* sequence alignment of *Colletotrichum* isolates from *Fragaria* and representative strains of the *C. dematium* species complex. Bootstrap support values of the MP analysis >70% and of the ML analysis >95% are shown at the nodes. *Colletotrichum chlorophyti* IMI 103806 was used as outgroup. Numbers of ex-type strains are indicated by an asterisk. Strains obtained or sequenced in this study are emphasised in bold font. Strain numbers are followed by substrate (host genus) and country of origin (NL = Netherlands, CR = Czech Republic).

ly most parsimonious trees were retained (length = 1062 steps, CI = 0.760, RI = 0.862, RC = 0.655, HI = 0.240). One of them is shown in Figure 2. The topology of these trees was similar, which was verified for a large section of trees. They differed in the position of taxa within subclades. The consensus tree of the ML analysis confirmed the tree topology obtained with parsimony. The bootstrap values of the two analyses generally agreed with each other. Isolate GLMC 2587 clustered with *C. lineola* strains within a big clade (MP/ML: 92/100), which consisted of several closely related species. Isolate GLMC 2616 clustered within *C. anthrisci* (MP/ML: 88/-), forming a sister clade to *C. sambucicola* (MP/ML: 100/100).

Taxonomy

As a result of the multi-locus molecular analyses, the 58 isolates were assigned to five species, of which *C. nymphaeae*, *C. godetiae* and *C. fioriniae* belong to the *C. acutatum* species complex; *C. anthrisci* and *C. lineola* are in the *C. dematium* species complex. Isolates of all these species were studied in culture, and their characteristics are outlined below.

Colletotrichum anthrisci Damm, P.F. Cannon & Crous, *Fungal Diversity* **39**: 56 (2009). (Figure 3)

Sexual morph not observed. *Asexual morph on SNA*. *Vegetative hyphae* 2–5.5 µm diam., hyaline, smooth-walled, septate, branched. *Conidiomata* acervular, conidiophores and setae formed on pale brown angular to roundish basal cells. *Setae* dark brown, 125–165 µm long, bases conical to slightly inflated, 11–15.5 µm diam., tapering to acute apices. *Conidiophores* hyaline to pale brown, smooth-walled, septate. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical to clavate, 8–14 × 2.5–3 µm, opening up to 2 µm diam.,

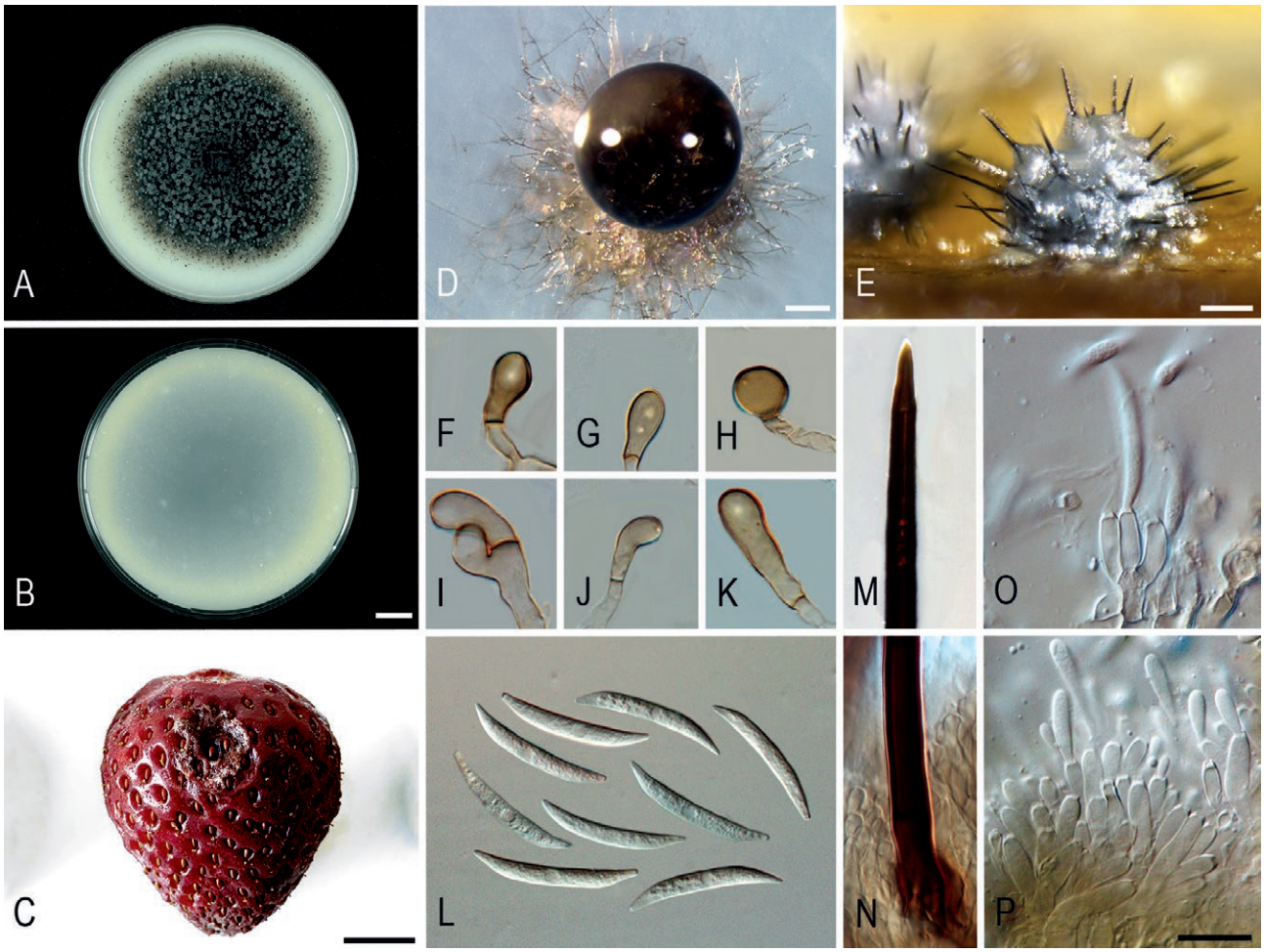

Figure 3. *Colletotrichum anthrisci* (strain GLMC 2616). A–B. Cultures on OA after 10 d. A. upper and B. reverse side. C. Symptom on fruit of *F.* × *ananassa* 'Asia' 10 dpi. D. Conidioma on SNA. E. Conidioma on *Anthriscus* stem. F–K. Appressoria. L. Conidia. M. Tip of a seta. N. Base of a seta. O–P. Conidiophores. F–P. from SNA. D–E. DM. F–P. DIC. Scale bars: B, C = 1 cm, D = 100 µm, E = 200 µm, P = 10 µm. Scale bar of B applies to A–B. Scale bar of P applies to F–P.

collarettes distinct, up to 2 µm long, periclinal thickening visible. *Conidia* hyaline, aseptate, smooth-walled, slightly curved, bases truncate, apices acute, (23.5–)25.5–27.5 × 2.5–3.5 µm, mean ± SD = 26.4 ± 1.0 × 3.0 ± 0.4 µm, L/W ratio = 8.8. *Appressoria* single, pale to medium brown, clavate to navicular, with entire edges, sometimes crenate, 5–22.5(–44) × (5–)5.5–8.5(–12) µm, mean ± SD = 13.7 ± 8.8 × 7.0 ± 1.6 µm, L/W ratio = 2.0. *Asexual morph on* Anthriscus *stems*. *Conidiomata* acervular, conidiophores and setae formed on pale brown angular cells. *Setae* dark brown, 122.5–155 µm long, bases conical to slightly inflated, 11.5–15.5 µm diam., tapering to acute apices. *Conidiophores* hyaline to pale brown, smooth-walled, septate. *Conidiogenous cells* hyaline to pale brown, smooth-walled, cylindrical to clavate, 12.5 × 3 µm, opening up to 2 µm diam., collarettes distinct, up to 2 µm long, periclinal thickening visible. *Conidia* hyaline, smooth-walled, aseptate, slightly curved, bases truncate, apices acute, (25–)26–27(–27.5) × 2.5–3.5 µm, mean ± SD = 26.5 ± 0.7 × 3.1 ± 0.4 µm, L/W ratio = 8.6.

Cultural characteristics. *Colonies on SNA* flat with entire margins, medium hyaline to pale cinnamon partly covered by whitish aerial mycelium, filter paper partly covered by olivaceous grey aerial mycelium, medium and *Anthriscus* stems partly with tiny dark grey spots, colony reverse sides same colours; 20–25 mm in 7 d (31–37 mm in 10 d). *Colonies on OA* flat with entire margins, olivaceous grey to iron grey, buff with iron grey spots towards the margins, colony reverse sides olivaceous grey, buff towards the margins; 18–26 mm in 7 d (30–35 mm in 10 d). Conidia in mass whitish to pale grey.

Material examined: Germany, Saxony, Markersdorf, forest, leaf spot of *Fragaria vesca*, 22 Sep. 2019, U. Damm, culture GLMC 2616 = DSM 115225.

Notes: Although the molecular data were identical, the conidia of the isolate from *F. vesca* formed on both media were slightly narrower, and appressoria were shorter (mean ± SD = 13.7 ± 8.8 × 7.0 ± 1.6 µm vs. 17.3 ± 6.1 × 7.0 ± 1.3 µm) than those in the original description of *C. anthrisci* (Damm *et al*., 2009).

Colletotrichum fioriniae (Marcelino & Gouli) Pennycook, *Mycotaxon* **132**(1): 150 (2017). (Figure 4)

Sexual morph not observed. *Asexual morph on SNA*. *Vegetative hyphae* 1.5–4 µm diam., hyaline, smooth-walled, septate, branched. *Conidiomata* acervular, conidiophores formed on pale brown, angular cells. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate. *Conidiogenous cells* hyaline, smooth-walled, cylindrical, 11–14 × 3 µm, openings 1–1.5 µm diam., collarettes up to 1 µm long. *Conidia* hyaline, aseptate, smooth-walled, fusiform, both ends acute, (12–)13.5–16.5(–17.5) × 4–5.5 µm, mean ± SD = 15.0 ± 1.5 × 4.6 ± 0.5 µm, L/W ratio = 3.3. *Appressoria* single, pale brown, mostly clavate, with entire edges, (5.5–)7–11(–14.5) × (4.5–)5–6.5(–8) µm, mean ± SD = 8.9 ± 2.1 × 5.8 ± 0.7 µm, L/W ratio = 1.5. *Asexual morph on* Anthriscus *stems*. *Conidiomata* acervular, conidiophores formed on pale brown, angular cells. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate. *Conidiogenous* cells hyaline, smooth-walled, cylindrical, 12–14 × 2.5–3 µm, openings up to 1.5 µm diam., collarettes distinct, up to 1 µm long, periclinal thickenings visible. *Conidia* hyaline, aseptate, smooth-walled, fusiform,

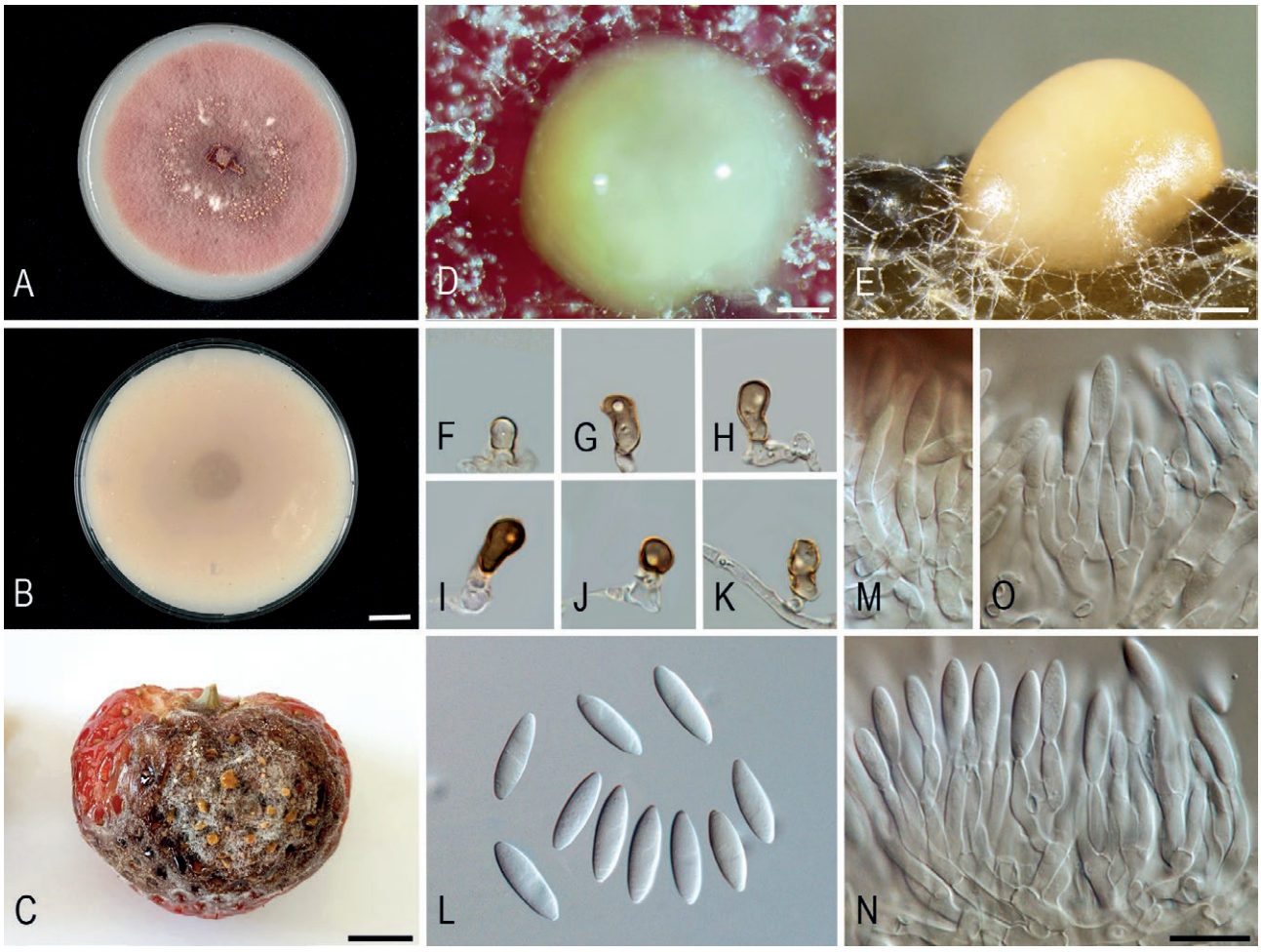

Figure 4. *Colletotrichum fioriniae* (strain GLMC 2660). A–B. Cultures on OA after 10 d. A. upper and B. reverse side. C. Symptom on fruit of *F.* × *ananassa* 'Asia' 7 dpi. D. Conidioma on SNA. E. Conidioma on *Anthriscus* stem. F–K. Appressoria. L. Conidia. M–O. Conidiophores. F–O. from SNA. D–E. DM. F–O. DIC. Scale bars: B, C = 1 cm, D–E = 100 µm, N = 10 µm. Scale bar of B applies to A–B. Scale bar of N applies to F–O.

both ends acute, (12.5–)14.5–16.5(–17.5) × 4–5.5 µm, mean ± SD = 15.4 ± 1.0 × 4.8 ± 0.4 µm, L/W ratio = 3.2.

Cultural characteristics. *Colonies on SNA* flat with entire margins, medium hyaline, pale cinnamon to pale peach, partly covered by whitish aerial mycelium, *Anthriscus* stems partly covered by saffron spore masses, colony reverse sides same colours; 21–26 mm in 7 d (30–38 mm in 10 d). *Colonies on OA* flat with undulate margins, surface coral to luteous, almost entirely covered by felty, flesh to rosy vinaceous aerial mycelium and saffron spore masses, colony reverse sides red, coral, brick to apricot; 9–18 mm in 7 d (14–30 mm in 10 d). Conidia in mass saffron.

Material examined: Germany, Saxony (Ore Mountains), from fruit anthracnose of *Fragaria* × *ananassa*, 18 Aug. 2021, C. Rose, culture GLMC 2660 = DSM 115228.

Notes: The morphology generally agreed with that of the ex-type strain on the same media. The isolate from strawberry studied here had very distinctly fusiform conidia, while other strains had fusiform to cylindrical conidia each with one round and one slightly acute end (Damm *et al*., 2012a).

Colletotrichum godetiae Neerg., *Friesia* **4**(1–2): 72 (1950). (Figure 5)

Sexual morph not observed. *Asexual morph on SNA*. *Vegetative hyphae* 2–4 µm diam., hyaline, smooth-walled, septate, branched. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate. *Conidiogenous cells* hyaline, smooth-walled, cylindrical, 11–18 × 3.5 µm, opening 1 µm diam., collarette up to 1.5 µm long, periclinal thickenings visible. *Conidia* hyaline, asep-

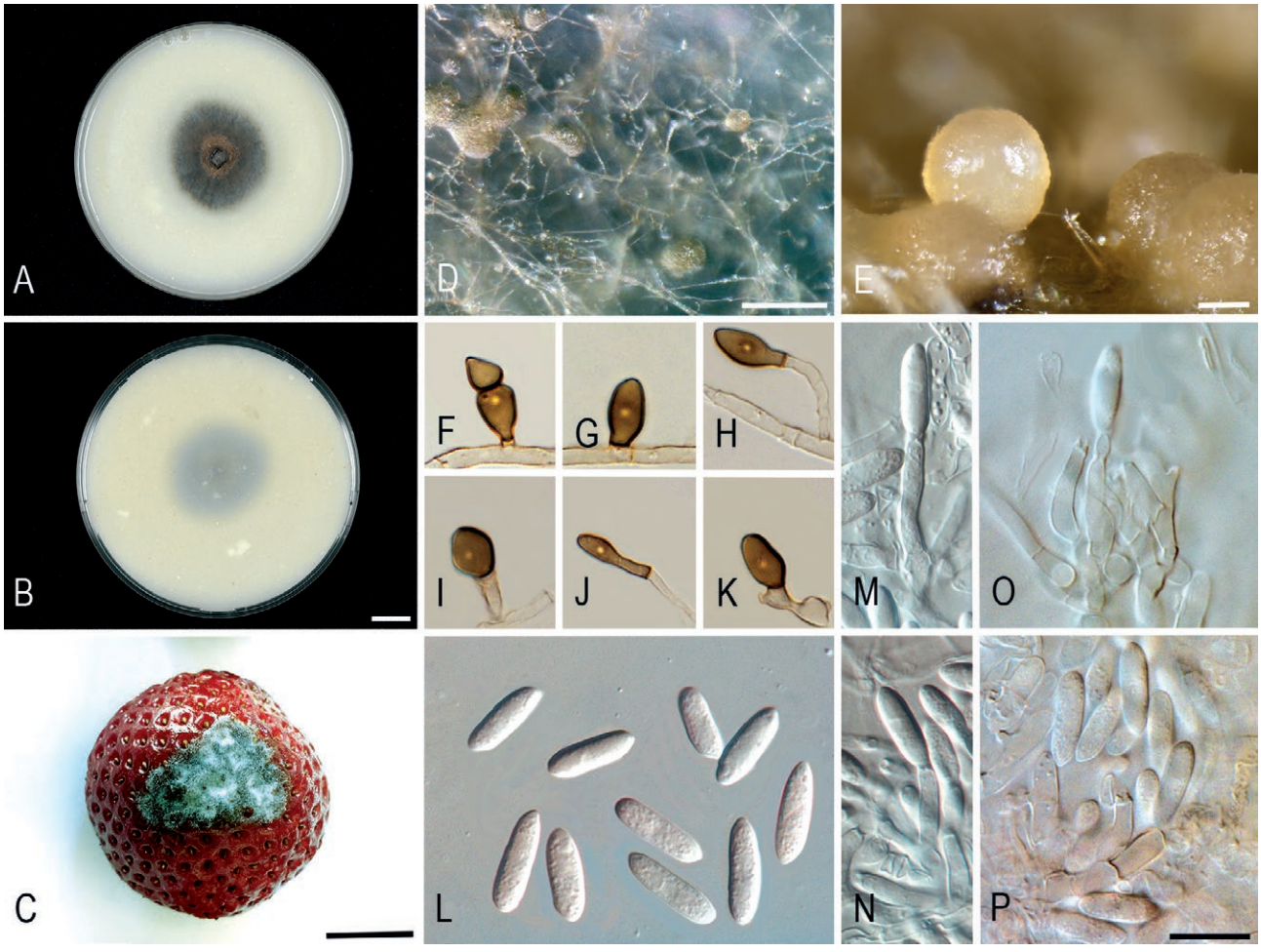

Figure 5. *Colletotrichum godetiae* (strain GLMC 2590). A–B. Cultures on OA after 10 d. A. upper and B. reverse side. C. Symptom on fruit of *F.* × *ananassa* 'Asia' 7 dpi. D. Conidiomata on SNA. E. Conidiomata on *Anthriscus* stem. F–K. Appressoria. L. Conidia. M–P. Conidiophores. F–P. from SNA. D–E. DM. F–P. DIC. Scale bars: B, C = 1 cm, D = 100 µm, E = 200 µm, P = 10 µm. Scale bar of B applies to A–B. Scale bar of P applies to F–P.

tate, smooth-walled, cylindrical, each one end round, the other end acute to round, (14.5–)15–16.5(–17.5) × (4–)4.5–5(–5.5) µm, mean ± SD = 15.9 ± 0.8 × 4.7 ± 0.3 µm, L/W ratio = 3.4. *Appressoria* medium brown, ellipsoidal to clavate, with entire to undulate edges, appressoria of strain GLMC 2590 measured (6–)7.5–13(–17.5) × (4–)4.5–6(–6.5) µm, mean ± SD = 10.3 ± 2.9 × 5.5 ± 0.7 µm, L/W ratio = 1.9, appressoria of GLMC 2589 broader, measuring (5–)7.5–13(–18) × (3.5–)5–7.5(–8.5) µm, mean ± SD = 10.0 ± 2.6 × 6.2 ± 1.2 µm, L/W ratio = 1.6. *Asexual morph on* Anthriscus *stems*. *Conidiomata* acervular, conidiophores on pale brown angular cells. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate. *Conidiogenous cells* hyaline, smooth-walled, cylindrical, 9.5–21 × 3 µm, openings 1.5 µm diam., collarettes up to 2 µm long, periclinal thickenings visible. *Conidia* hyaline, aseptate, smooth-walled, cylindrical, each one end round, the other end acute to round, conidia of GLMC 2590 measured (15–)15.5–17(–17.5) × 4.5–5 µm, mean ± SD = 16.0 ± 0.8 × 4.7 ± 0.3 µm, L/W ratio = 3.4, conidia of GLMC 2589 shorter, measuring (12.5–)13.5–15.5(–17) × (4–)4.5–5 µm, mean ± SD = 14.7 ± 1.1 × 4.6 ± 0.2 µm, L/W ratio = 3.2.

Cultural characteristics. *Colonies on SNA* flat with entire margins, medium hyaline to pale cinnamon, partly covered by whitish to pale grey aerial mycelium, *Anthriscus* stems partly covered by saffron spore masses, filter papers partly pale olivaceous grey to olivaceous grey, colony reverse sides same colours; GLMC 2590: 18–24 mm in 7 d (29–35 mm in 10 d). *Colonies on OA* flat with entire margins, covered by woolly to felty, greyish sepia, pale smoke grey to white aerial mycelium, colony reverse sides pale purplish grey vinaceous grey to fuscous black; GLMC 2590: 6–19 mm in 7 d (15–28 mm in 10 d), GLMC 2589 faster growing: 16–22 mm in 7 d (25–32 mm in 10 d). Conidia in mass saffron.

Material examined: Germany, Mecklenburg-Western Pomerania, Rostock, vegetable farm, from a fruit of *Fragaria* × *ananassa*, 17 June 1999, P. Steinbach, culture GLMC 2590 = BBA 71234 = DSM 115223; Baden-Württemberg, from a necrotic runner of *Fragaria* × *ananassa*, unknown collection date (accessed by BBA 31 Oct. 1996), culture GLMC 2589 = BBA 70063 = DSM 115222.

Notes: Sequence data and morphology of *C. godetiae* are very variable, including the shape and size of the conidia that are studied and discussed in Damm *et al.* (2012a). While the ex-type strain and a strain from *Fragaria* in the Netherlands (CBS 125972) formed comparatively fusiform conidia, conidia of other strains, including those from *Fragaria* in Germany studied here, were rather cylindrical, and those of further strains were even clavate (Damm *et al.*, 2012a; this study, Figure 5L).

Colletotrichum lineola Corda, *Deutschlands Flora*, Abt. III. Die Pilze Deutschlands **3** (12): 41 (1831). (Figure 6)

Sexual morph not observed. *Asexual morph on SNA*. *Vegetative hyphae* 3.5–5 µm diam., hyaline, smooth-walled, septate, branched. *Conidiomata* acervular, conidiogenous cells and setae formed on bases of brown angular cells. *Setae* dark brown, 64–72.5 µm long, bases cylindrical to slightly inflated, 2.5–5 µm diam. *Conidiophores* pale brown, smooth-walled, septate. *Conidiogenous cells* pale brown, smooth-walled, cylindrical, 12–16.5 × 3.5 µm, openings 1–1.5 µm diam., collarettes visible. *Conidia* hyaline, aseptate, smooth-walled, slightly curved, bases truncate, apices acute, (21.5–)22.5–25.5(–26.5) × 2.5–3.5 µm, mean ± SD = 24.1 ± 1.4 × 3.1 ± 0.4 µm, L/W ratio = 7.7. *Appressoria* single, medium brown, obovoidal to clavate, sometimes crenate or lobed, (5–)6.5–16.5(–23) × 4.5–11(–16) µm, mean ± SD = 11.6 ± 4.9 × 7.9 ± 3.2 µm, L/W ratio = 1.5. *Asexual morph on* Anthriscus *stems*. *Conidiomata* acervular, conidiophores formed on brown angular cells. *Setae* dark brown, 64.5–68.5 µm long, base 2–4.5 µm diam. *Conidiophores* pale brown, smooth-walled, septate. *Conidiogenous cells* pale brown, smooth-walled, cylindrical, 13.5–16.5 × 3.5 µm, openings 1.5 µm diam. *Conidia* hyaline, aseptate, smooth-walled, slightly curved, bases truncate, apices acute, (22–)23–26(–26.5) × (2.5–)3–3.5 µm, mean ± SD = 24.4 ± 1.4 × 3.1 ± 0.3 µm, L/W ratio = 7.8.

Cultural characteristics. *Colonies on SNA* flat with entire margins, medium hyaline, pale cinnamon to pale ochreous, medium, filter papers and *Anthriscus* stems partly covered by tiny olivaceous black spots, aerial mycelium lacking, colony reverse sides same colours; 29–36 mm in 7 d (38–>40 mm in 10 d). *Colonies on OA* flat with entire margins, medium buff to honey, with tiny olivaceous grey to olivaceous black spots, aerial mycelium lacking, colony reverse sides honey to vinaceous buff; 21–26 mm in 7 d (36–>40 mm in 10 d). Conidia in mass whitish.

Material examined: Germany, Brandenburg, from fruit anthracnose of *Fragaria* × *ananassa*, 19 Jun. 2001, unknown collector, culture GLMC 2587 = BBA 71830 = DSM 115221.

Notes: The morphology of isolate GLMC 2587 agreed with the description in Damm *et al.* (2009) that based on material from *Apiaceae* hosts, except for the slightly longer conidia of the isolate from strawberry formed on *Anthriscus* stems.

Colletotrichum nymphaeae (Pass.) Aa, *Netherlands J. Plant Pathol.* **84**: 110 (1978). (Figure 7)

Sexual morph not observed. *Asexual morph on SNA*. *Vegetative hyphae* 2–3.5 µm diam., hyaline, smooth-

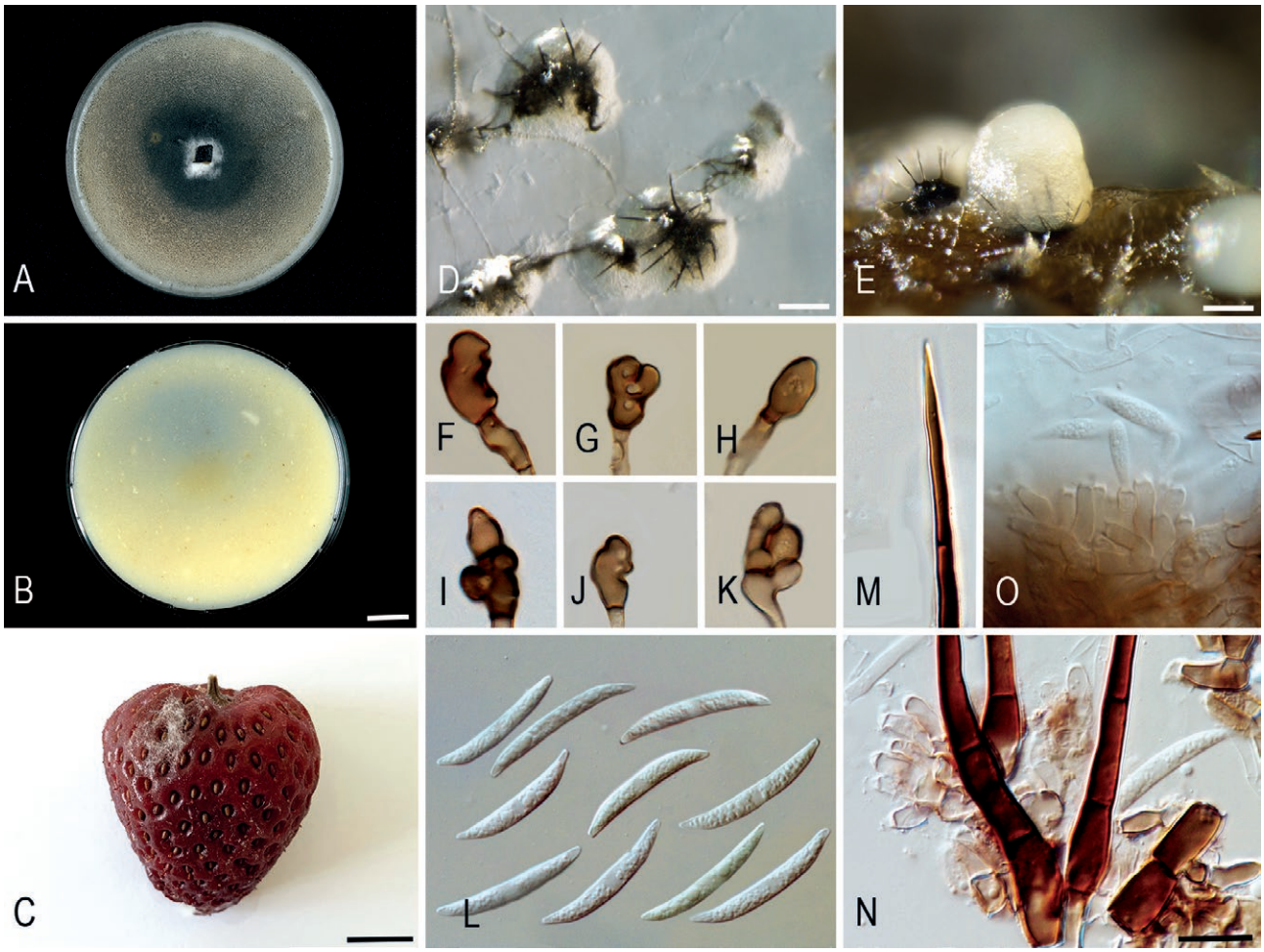

Figure 6. *Colletotrichum lineola* (strain GLMC 2587). A–B. Cultures on OA after 10 d. A. upper and B. reverse side. C. Symptom on fruit of *F.* × *ananassa* 'Asia' 10 dpi. D. Conidiomata on SNA. E. Conidiomata on *Anthriscus* stem. F–K. Appressoria. L. Conidia. M. Tip of a seta. N. Bases of setae. O. Conidiophores. F–O. from SNA. D–E. DM. F–O. DIC. Scale bars: B, C = 1 cm, D, E = 200 µm, N = 10 µm. Scale bar of B applies to A–B. Scale bar of N applies to F–O.

walled, septate, branched. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate. *Conidiogenous cells* hyaline, smooth-walled, cylindrical, 14–15.5 × 2–3 µm, openings 1.5–2 µm diam., collarettes up to 2 µm long, periclinal thickenings distinct. *Conidia* hyaline, aseptate, smooth-walled, cylindrical to fusiform, ends acute to round, conidia of GLMC 2445 measured (8.5–)12–16(–17) × (2–)2.5–3.5(–4.5) µm, mean ± SD = 13.8 ± 1.9 × 3.1 ± 0.5 µm, L/W ratio = 4.5, conidia of GLMC 2653 shorter and wider, measuring (4.5–)9–15.5(–18) × (3–)3.5–4.5(–5) µm, mean ± SD = 12.4 ± 3.3 × 3.9 ± 0.5 µm, L/W ratio = 3.2, conidia of GLMC 2656 longer, measuring (8.5–)12–17(–22.5) × (2.5–)3.5–4(–4.5) µm, mean ± SD = 14.7 ± 2.5 × 3.7 ± 0.3 µm, L/W ratio = 4.2. *Appressoria* single, medium to pale brown, mostly clavate, with entire edges, appressoria of GLMC 2445 measured (6.5–)7–13(–20.5) × (5.5–)6–7.5(–9) µm, mean ± SD = 10.0 ± 3.2 × 6.8 ± 0.6 µm, L/W ratio = 1.5, appressoria of GLMC 2556 narrower, measuring (5–)6.5–13(–21) × (3.5–)5–7(–9) µm, mean ± SD = 10.0 ± 3.3 × 6.0 ± 1.1 µm, L/W ratio = 1.7. *Asexual morph on* Anthriscus *stems*. *Conidiomata* acervular, conidiophores formed on brown angular cells. *Setae* not observed. *Conidiophores* hyaline, smooth-walled, septate. *Conidiogenous cells* hyaline, smooth-walled, cylindrical, 14.5–18 × 2 µm, openings 1.5–2 µm diam., collarettes distinct, up to 2 µm long, periclinal thickenings visible. *Conidia* hyaline, aseptate, smooth-walled, fusiform to cylindrical, ends acute to round. (13.5–)14.5–17(–18.5) × 4–4.5 µm, mean ± SD = 15.7 ± 1.3 × 4.3 ± 0.2 µm, L/W ratio = 3.6, conidia of GLMC 2653 shorter, measuring (9.5–)12–14.5(–16) × (3–)4–4.5 µm, mean ± SD = 13.3 ± 1.3 × 4.3 ± 0.4 µm, L/W ratio = 3.1.

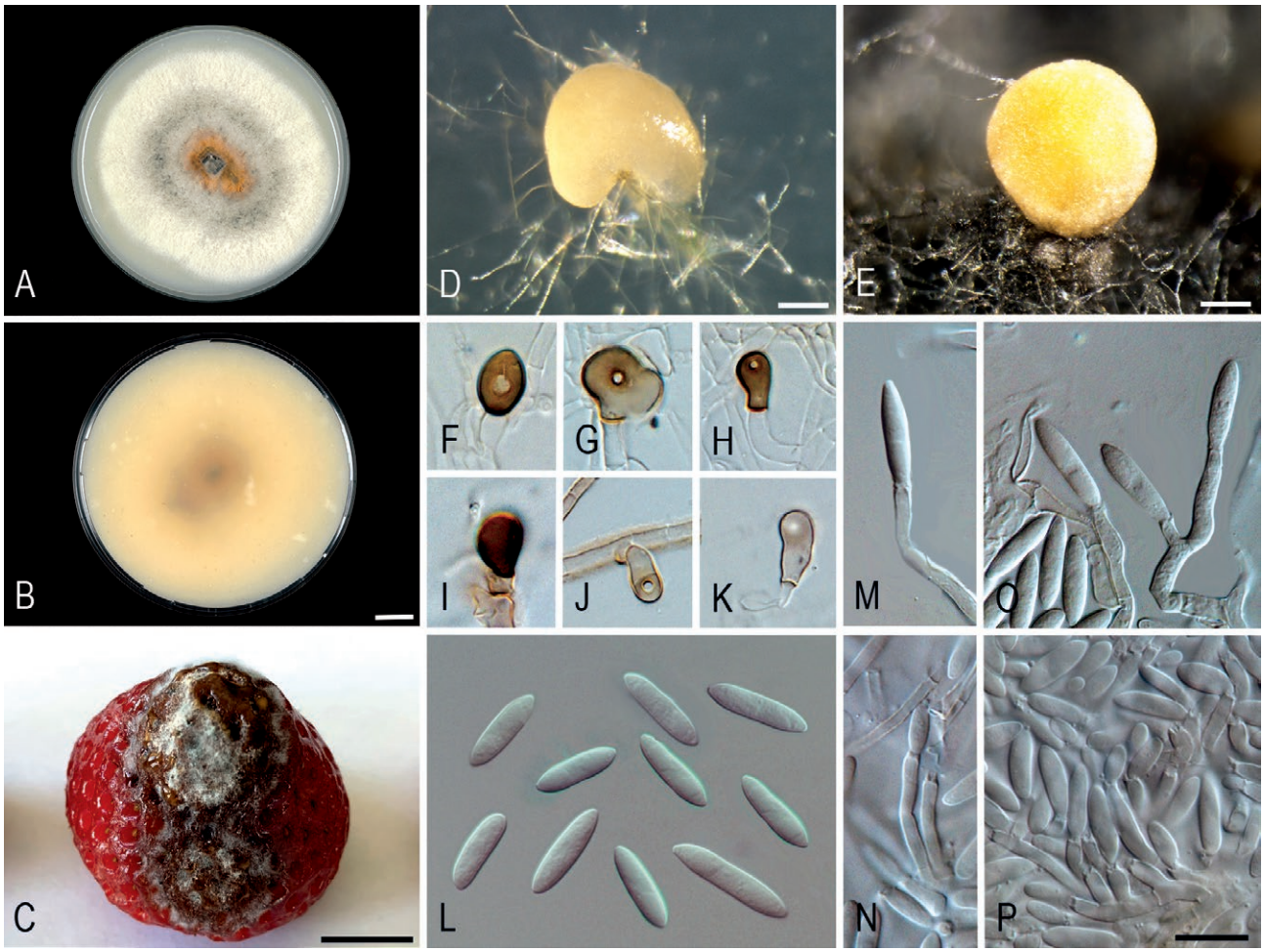

Figure 7. *Colletotrichum nymphaeae* (strain GLMC 2445). A–B. Cultures on OA after 10 d. A. upper and B. reverse side. C. Symptom on fruit of *F.* × *ananassa* 'Asia' 7 dpi. D. Conidioma on SNA. E. Conidioma on *Anthriscus* stem. F–K. Appressoria. L. Conidia. M–P. Conidiophores. F–P. from SNA. D–E. DM. F–P. DIC. Scale bars: B, C = 1 cm, D = 200 µm, E = 100 µm, P = 10 µm. Scale bar of B applies to A–B. Scale bar of P applies to F–P.

Cultural characteristics. Colonies on SNA flat with entire margins, medium hyaline to pale cinnamon, partly covered by whitish aerial mycelium and by saffron to orange spore masses, filter paper partly pale olivaceous grey, colony reverse sides hyaline to pale cinnamon, saffron to orange spore masses shining through, filter paper partly pale olivaceous grey to olivaceous black; 19–30 mm in 7 d (31–36 mm in 10 d). *Colonies on OA* flat with entire margins, entirely covered with woolly to felty, greyish sepia, pale mouse grey, rosy buff to white aerial mycelium, orange spore masses mainly in the centres, colony reverse sides olivaceous grey, saffron to pale luteous; GLMC 2445 16–25 mm in 7 d (29–39 mm in 10 d), GLMC 2656 slower growing: 16–22 mm in 7 d (25–32 mm in 10d). Conidia in mass saffron to orange.

Material examined: Germany, Saxony, Dresden, from fruit anthracnose of *F.* × *ananassa* 'Asia', 1 Jul. 2019, C. Rose, culture GLMC 2445 = DSM 115220; North Rhine Westphalia, near Münster, from brown spots on petiole of *F.* × *ananassa* 'Asia', 10 Sep. 2021, C. Rose, culture GLMC 2656; North Rhine Westphalia, near Münster, from brown spots on leaf of *F.* × *ananassa* 'Asia', 10 Sep. 2021, C. Rose, culture GLMC 2658; Brandenburg, Spreewald (bought on a market in Dresden), from fruit anthracnose of *F.* × *ananassa*, 16 Sep. 2021, C. Rose, culture GLMC 2653 = DSM 115226.

Notes: The ex-type strain of *C. nymphaeae* formed clavate conidia, while other strains studied by Damm *et al.* (2012a) including strains from *Fragaria* as well as the strains from *Fragaria* in Germany studied here form fusiform to cylindrical conidia.

Pathogenicity

Seven dpi, all tested isolates of the five *Colletotrichum* species caused symptoms on strawberry fruit *F.* × *ananassa* 'Asia'. However, for almost all isolates and species in both experiments, some fruit did not develop symptoms or showed symptoms that were very slight 7 dpi.

Colletotrichum anthrisci and *C. lineola* caused no or small lesions 7 dpi (GLMC 2616: 0.0–0.25 cm^2, GLMC 2587: 0.0–0.5 cm^2). Even 10 dpi the symptoms were small: after inoculations with *C. anthrisci*, circular, dry, sunken, black necroses developed that contained tiny dark grey spots, covered by whitish aerial mycelium and rosy vinaceous conidial masses (Figure 3C, 10 dpi). After inoculations with *C. lineola*, circular, dry, brown, sunken necroses developed, which were covered by sparse whitish aerial mycelium; rosy vinaceous conidial masses developed (Figure 6C, 10 dpi). The two strains of *C. godetiae* developed noticeable fruit symptoms 7 dpi. Variability of lesion sizes from the two strains was similar (GLMC 2589: 0.2–1.2 cm^2, GLMC 2590: 0.5–1.0 cm^2). Necroses formed that were nearly circular, brown, sunken and entirely covered by uniform woolly to felty, white to pale smoke grey aerial mycelium, without conidium formation (Figure 5C, 7 dpi).

Variability of strawberry lesion sizes was high after inoculations with the two isolates of *C. fioriniae*, and the nine isolates of *C. nymphaeae*. Both species caused large necrotic areas with abundant sporulation. The isolates of *C. fioriniae* formed circular, dry, dark brown, sunken necrotic areas, almost entirely covered by felty whitish to grey mycelium and saffron to brick conidial masses that were aggregated to large drops. There were also drops of discharged dark liquid in the lesions (Figure 4C, 7 dpi). The lesion sizes 7 dpi were 0.1–2.4 cm^2 with isolate GLMC 2660 and 0.0–0.6 cm^2 with isolate GLMC 2661. *Colletotrichum nymphaeae* formed brown, dry, sunken necrotic areas on inoculated strawberries that were entirely covered by woolly to felty, white to pale mouse grey aerial mycelium in concentric rings and small orange conidial masses, as well as drops of discharged liquid (Figure 7C, 7 dpi). The lesion sizes 7 dpi with different isolates were: GLMC 2445: 0.5–1.5 cm^2, GLMC 2552: 0.12–0.95 cm^2, GLMC 2588: 0.02–2.5 cm^2, GLMC 2595: 0.0–0.1 cm^2, GLMC 2600: 0.05–1.75 cm^2, GLMC 2610: 1.5–2.75 cm^2, GLMC 2653: 0.0–0.95 cm^2, GLMC 2656: 0.0–0.5 cm^2 and GLMC 2658: 0.0–0.25 cm^2.

Due to infection by other fungi or collapsing of individual strawberries, some were excluded from both experiments, and resulted in failures, especially after inoculations with *C. nymphaeae* isolates GLMC 2445, GLMC 2588, GLMC 2600, GLMC 2610, GLMC 2658, *C. lineola* isolate GLMC 2587 and *C. fioriniae* isolate 2661. However, all inoculated fruit in the experiments showed symptoms 10 dpi.

The species that were inoculated were respectively re-isolated from all symptoms developing on inoculated strawberries. No symptoms developed after control treatments with sterile, distilled water.

DISCUSSION

This study has demonstrated that *Colletotrichum* is widespread on strawberries in Germany, although neither the BBA collection nor recent sampling described here covered all regions of this country. The *Colletotrichum* collection of the BBA shows occurrence of *Colletotrichum* on strawberries around the year 2000 in up to seven federal states of Germany, including Brandenburg, Lower Saxony, Saxony, Mecklenburg-Western Pomerania, Baden-Württemberg, Hesse and (probably) Rhineland-Palatinate. The new collections (2019–2021) confirm this genus in Brandenburg, Lower Saxony, Saxony, Mecklenburg-Western Pomerania, and also include collections from North Rhine-Westphalia.

Most of the samples in these collections were of *C. nymphaeae*, which is the most common *Colletotrichum* species on strawberry in Germany. This species belongs to the *C. acutatum* species complex, occurs on several hosts and is common on strawberry in Europe, Iran and North America (Damm *et al*., 2012a; Baroncelli *et al*., 2015; Karimi *et al*., 2017; Grammen *et al*., 2019; Wang *et al*., 2019; Tsvetkova and Kuznetsova, 2022). Based on the present study, *C. nymphaeae* is present in all federal states of Germany for which data are available and occurs on *F.* × *ananassa* and *F. vesca* var. *semperflorens*, a cultivated variety of wild strawberry. *Colletotrichum nymphaeae* was mostly isolated from fruit of *F.* × *ananassa* in the field and from the market. Fruit bought from the marked in Görlitz originated from Poland, confirming a recent report of *C. nymphaeae* on strawberries in that country (Tsvetkova and Kuznetsova, 2022). This species was also isolated from symptoms on different green plant parts of *F.* × *ananassa*, both from established plants in the field and from purchased young plants from German propagation culture.

In a survey of the *C. acutatum* species complex on strawberry in the USA, *C. nymphaeae* dominated (97.7% of the isolates), and almost all isolates were of one clonal lineage regardless of the isolation source (Wang *et al*., 2019). One representative of these strains (isolate 16-320) was included in the phylogeny of the present study, and this isolate grouped with most of the isolates

from strawberry and with the majority of those examined by Damm *et al.* (2012a) and Baroncelli *et al.* (2015). Thus, this one clonal lineage is almost entirely restricted to strawberry, is distributed throughout Europe, also occurs in Israel and some African countries, and is demonstrated here to occur in Germany and Poland and to be the dominating lineage in Germany, both in historical and recent collections (Damm *et al*., 2012a; the present study). Wang *et al*. (2019) suggested that this clonal lineage had been distributed throughout the USA and Canada via quiescently infected strawberry transplants, which could also explain its widespread occurrence in European countries. In contrast, the dominating species on strawberry in China, Korea, Japan and Taiwan belong to the *C. gloeosporioides* complex. The species detected in Korea, Japan and Taiwan were completely different from those found in Europe, while in China *C. nymphaeae* is also present, but to a lesser extent (Nam *et al*., 2013; Han *et al*., 2016; Jayawardena *et al*., 2016; Gan *et al*., 2017; Chung *et al*., 2020).

The other two haplotypes of *C. nymphaeae* from strawberry in Germany detected in the present study were also identical to haplotypes from the USA, as described by Wang *et al.* (2019). This indicates three possibly independent introductions of this pathogen to German strawberries. One of these two haplotypes represented a clone that is closely related to the most common haplotype, which also includes some strains from strawberry from other European countries. The German isolates were part of the old collection. In contrast, the other haplotype has several nucleotide differences to the other haplotypes occurring on strawberry and included only three isolates, one from strawberry in the USA and two from a recent collection on strawberry in Brandenburg, Germany. This could represent a new lineage of *C. nymphaeae* which has restricted distribution and unknown impacts. Despite its dominating incidence on strawberries, there are few records of *C. nymphaeae* in Germany prior to the present study, including a collection from Freyburg, Saxony-Anhalt by H. Jage and one from a dried-up pond close to Flemsdorf, Brandenburg by J. Kruse (Dämmrich *et al*., 2023). Both collections are from *Nymphaea*, and both specimens are kept in the fungarium of the Senckenberg Museum of Natural History Görlitz (GLM-F127296, GLM-F129560). However, these identifications were not confirmed by DNA sequence data.

Colletotrichum godetiae also belongs to the *C. acutatum* species complex and occurs on several hosts including many woody plants as well as strawberry (Damm *et al*., 2012a). Although this fungus is probably common on strawberry in Europe (Damm *et al*., 2012a; Baroncelli *et al*., 2015; Tsvetkova and Kuznetsova, 2022), it was found only twice on strawberries from Germany in the present study and was not detected in the new collections. Despite proven infectivity of *C. godetiae* on *F.* × *ananassa* 'Asia' under laboratory conditions, there has been no invasive spread in German strawberry stocks within the last 20 years. In contrast to *C. nymphaeae* and *C. fioriniae*, *C. godetiae* is not known from strawberry in the USA and Canada (Baroncelli *et al*., 2015; Wang *et al*., 2019). The only North American strain of this species included in Damm *et al.* (2012a) was from a different host, and the haplotype represented by the strains from strawberry in the present study occurs both on strawberry and on other hosts. This suggests spread of this species on strawberries within Europe, rather than an introduction from the USA or occasional transmissions to strawberries by other hosts. This species was previously found in Germany, on leaf spots of *Mahonia aquifolium* and in necrotic wood of sour cherry (*Prunus cerasus*) (Damm *et al*., 2012a; Bien and Damm, 2020). However, the present report is the first from strawberry in Germany. A clade that was previously regarded as a subclade of *C. godetiae* and comprised exclusively strains from South America was recently described as new species, *C. americanum* (Zapata *et al*., 2024).

Colletotrichum fioriniae is another species belonging to the *C. acutatum* species complex. This pathogen occurs on several hosts, mainly crops, and is common on fruit of apple and strawberry as well as *Vaccinium* species in Europe, the USA and New Zealand and on *Persea* in Australia. In Germany, *C. fioriniae* has previously only been reported from an indoor collection of *Grevillea* sp. (*Proteaceae*) (Damm *et al*., 2012a), an exotic genus from Australia. However, the present report is the first of *C. fioriniae* from strawberry in Germany, and is also the first report from an outdoor cultivated crop in Germany. In contrast to *C. nymphaeae*, the haplotype of *C. fioriniae* detected in the present study, is not specialised to strawberry. There are several haplotypes of this species that occur both on strawberry and other hosts (Damm *et al*., 2012a; this study). This suggests a different transmission route of this pathogen to strawberries, possibly by host-jumps rather than in planting material of the same host. The high variability between isolates of *C. fioriniae* from the same hosts could also be related to recombination, as the formation of sexual morphs and of hybrids with *C. acutatum* (*sensu stricto*) are known (Marcelino *et al*., 2008; Damm *et al*., 2012a). In the USA, *C. fioriniae* was determined to be of low incidence on strawberries (five of 217 *Colletotrichum* isolates; Wang *et al*., 2019). In the present study, *C. fioriniae* was isolated

only twice from recently collected material from one strawberry field. However, since this fungus was able to infect *F.* × *ananassa* 'Asia' under laboratory conditions with development of large necrotic areas and abundant sporulation, there is a potential risk of the pathogen spreading to other strawberry stocks.

As the two subclades of *C. fioriniae* reported in the phylogeny of Damm *et al.* (2012a) were not supported and had similar hosts and distributions, they were treated as one species. The phylogeny of Chen *et al.* (2022) that included new strains from *Malus domestica* in China supported the two subclades, and the second subclade was described as *C. orientale* [actually as "orientalis"], but invalidly (Art. 40.8 Shenzhen), while Zhang *et al.* (2023a) described two further related strains from China as *C. radermacherae*. Since new strains from ornamental plants in China were intermediate between all three species, Zhang *et al.* (2023b) reduced both new species to synonymy with *C. fioriniae*. In the phylogeny of the present study that included one "*C. radermacherae*" strain (GZCC 21-0814) but not the intermediate strains of Zhang *et al.* (2023b), the two subclades were supported by one analysis (ML) that was not applied in Damm *et al.* (2012a). The strains from strawberry in Germany belong to the subclade containing the ex-type strain of *C. fioriniae*.

Colletotrichum lineola belongs to the *C. dematium* species complex and had been isolated from dead plant parts and diseases of several plants, mainly in Central Europe and North America. These hosts included *Fragaria* (petiole) in Canada (Damm *et al.*, 2009). Tsvetkova and Kuznetsova (2022) also isolated *C. lineola* from strawberries in Russia. In the present study, this fungus was found on strawberry fruit in Germany for the first time. In the pathogenicity tests, this species also caused very small symptoms on fruit, and it was not detected in the new collections. Therefore, *C. lineola* can be regarded as of minor importance for strawberry cultivation.

Another species of the *C. dematium* complex, *C. anthrisci*, was described from dead stems of *A. sylvestris* in the Netherlands (Damm *et al.*, 2009) and was recently classified as highly endangered (Talhinhas and Baroncelli, 2021). In contrast, this fungus was found to be ubiquitous in a forest in Japan, where it was isolated from seedlings of several trees, including *Prunus grayana*, *Fraxinus lanuginosa*, *Cornus controversa* and *Magnolia obovata* that had been killed by damping-off (Konno *et al.*, 2011). *Colletotrichum anthrisci* was also recorded on avocado fruit with anthracnose symptoms in Chile and was confirmed to cause this disease (Bustamente *et al.*, 2022). In the present study, *C. anthrisci* was isolated from leaf spots of *F. vesca* in a forest in Germany and was shown to cause fruit anthracnose of cultivated strawberry. Thus, this species is neither host-specific nor rare, and is known from three continents where it is at least locally very common. This is the first report of *C. anthrisci* both from Germany and from *Fragaria* worldwide and the first evidence of this species causing anthracnose on cultivated strawberry fruit under laboratory conditions. However, the symptoms caused on *F.* × *ananassa* 'Asia' were mild and *C. anthrisci* has to date not been collected from cultivated strawberry in the field.

Prior to the present study, most of the *Colletotrichum* strains from strawberry in Europe had been identified as *C. acutatum*. This also applies to the isolates from the BBA collection examined, one of which was the basis of the report by Nirenberg *et al.* (2002). In the present study, isolates previously identified as *C. acutatum* were re-identified as *C. nymphaeae*, except for the isolate originating from the Netherlands that was shown to be *C. godetiae*. None of the isolates were *C. acutatum* (*sensu stricto*). *Colletotrichum acutatum* (*sensu stricto*) has been found predominantly in the southern hemisphere (Damm *et al.*, 2012a) and is known to be associated with fruit rot of strawberry, but only in Australia (Sreenivasaprasad and Talhinhas, 2005; Damm *et al.*, 2012a). To date, there is no confirmed occurrence of *C. acutatum* (*sensu stricto*) on strawberries elsewhere in the world. Nearly all reports from strawberry prior to the treatment of the *C. acutatum* complex by Damm *et al.* (2012a) and some later reports, refer to other species within this complex, which was confirmed here for the pathogens causing strawberry anthracnose in Germany.

Other previously unreported *Colletotrichum* strains from the BBA collection had been identified as *C. truncatum*, *C. fragariae* and *C. gloeosporioides*, based on the original strain list. In the present study, *C. truncatum* was re-identified as *C. lineola*, and *C. fragariae* and *C. gloeosporioides* were re-identified as *C. godetiae*. *Colletotrichum truncatum* has not been reported from Germany (Farr and Rossman, 2024). This fungus forms curved conidia, but with different shape than *C. lineola* and belongs to the *C. truncatum* species complex, while *C. lineola* belongs to the *C. dematium* complex (Damm *et al.*, 2009). In contrast, *C. fragariae* and *C. gloeosporioides* form cylindrical conidia and belong to the *C. gloeosporioides* complex. However, no species of the *C. gloeosporioides* complex were found among the isolates from Germany that were examined here. However, the conidia of the *C. godetiae* isolates from strawberry studied here were more cylindrical than fusiform (Figure 5L). The cylindrical conidium shape of isolates of *C. godetiae*, like those from strawberries studied here, as well as of some other species of the *C. acutatum* species complex, is reminiscent of species of the *C. gloeosporioides* complex

(Damm *et al*., 2012a; Weir *et al*., 2012). This can cause confusion because identifications of isolates by morphology only are not reliable, even to species complex level. This also applies to species with curved conidia, as for the *C. lineola* isolate examined in this study. In contrast, *C. theobromicola* (syn. *C. fragariae*) has only been reported from strawberries from a few countries, of which only isolates from the USA have been confirmed by sequence data, while sequences of *C. fragariae* reported from the United Kingdom and Japan suggest also *C. godetiae* and a different species in the *C. gloeosporioides* complex, respectively (Nirenberg *et al*., 2002; Moriwaki *et al*., 2003; Weir *et al*., 2012; Farr and Rossman, 2024). The only reliable reports of *C. gloeosporioides* (*sensu stricto*) from strawberries are from China (e.g. Han *et al*., 2016). As the present study was the first molecular identification of *Colletotrichum* strains from strawberries in Germany after the revisions of the respective *Colletotrichum* species complexes based on multi-locus sequence data (Damm *et al*., 2009, 2012a; Weir *et al*., 2012), all species detected in this study are new reports on strawberries in Germany.

While symptoms caused by *C. eriobotryae* and *C. nymphaeae* on loquat fruit were indistinguishable (Damm *et al*., 2020), those caused by the species tested on strawberry fruit in the present study were very different from each other. This could be attributed to the high genetic distance of the species studied here that belong to different species complexes or at least different main clades within the *C. acutatum* complex. The two species from loquat both belonged to clade 2 of the *C. acutatum* complex. Although the symptoms caused by the five different species were typical for the individual species, it is unlikely that species can be identified based on host symptoms alone.

Colletotrichum nymphaeae and *C. fioriniae* were more aggressive than *C. godetiae* in strawberry fruit assays reported by Baroncelli *et al.* (2015). This can be tentatively confirmed by the present study, although our data could not be statistically analysed due to large variations and nil results. In pathogenicity tests, MacKenzie *et al.* (2009) showed that *Colletotrichum* isolates from strawberry and blueberry from Florida (USA) that were later identified as *C. nymphaeae* and *C. fioriniae*, respectively, based on ITS and *gapdh* sequences (Damm *et al*., 2012a), caused anthracnose on strawberry fruits; the lesions caused by *C. nymphaeae* isolates were larger than those caused by *C. fioriniae* isolates. This cannot be confirmed by the present study, because lesion sizes caused by one of the *C. fioriniae* isolates belonged to the largest in the tests. Because of variability in lesion sizes caused by these two species, no conclusions can be drawn about relative virulence of these species. Based on the number of isolates and virulence, *C. nymphaeae* is likely to be the most important strawberry anthracnose pathogen in Europe, representing the highest economic risk for commercial strawberry production. This was previously confirmed for the United Kingdom and Russia and in the present study also for Germany.

The present study has identified the pathogens causing anthracnose of cultivated strawberry in Germany, which provides the basis for application and development of targeted control measures for management of these pathogens in commercial strawberry cultivation. This will require assessments of fungicide effectiveness and pathogen resistance. Targeted strawberry breeding should be aimed at resistance to specific *Colletotrichum* pathogens defined at species and/or haplotype level. Monitoring of pathogens in the field and testing of acquired, especially imported planting material using molecular methods would help to detect possible new pathogen species and haplotypes, as the pathogen spectrum could change due to changes in prevailing climates.

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