Abstract: *Colletotrichum* is regarded as one of the 10 most important genera of plant pathogens in the world. It causes diseases in a wide range of economically important plants, including peaches. China is the largest producer of peaches in the world but little is known about the *Colletotrichum* spp. affecting the crop. In 2017 and 2018, a total of 286 *Colletotrichum* isolates were isolated from symptomatic fruit and leaves in 11 peach production provinces of China. Based on multilocus phylogenetic analyses (ITS, ACT, CAL, CHS-1, GAPDH, TUB2, and HIS3) and morphological characterization, the isolates were identified to be *C. nymphaeae*, *C. fioriniae*, and *C. godetiae* of the *C. acutatum* species complex, *C. fructicola* and *C. siamense* of the *C. gloeosporioides* species complex, and one newly identified species, *C. folicola* sp. nov. This study is the first report of *C. karsti* and *C. godetiae* in peaches, and the first report of *C. nymphaeae*, *C. fioriniae*, *C. fructicola*, and *C. siamense* in peaches in China. *C. nymphaeae* is the most prevalent species of *Colletotrichum* in peaches in China, which may be the result of fungicide selection. Pathogenicity tests revealed that all species found in this study were pathogenic on both the leaves and fruit of peaches, except for *C. folicola*, which only infected the leaves. The present study substantially improves our understanding of the causal agents of anthracnose on peaches in China.

Keywords: *Colletotrichum*; peach anthracnose; multilocus phylogeny; pathogenicity; taxonomy

1. Introduction

The peach (*Prunus persica* (L.) Batsch) originated in China [1] and has been grown in many temperate climates around the world. Published: 18 March 2022

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).
mummies and affected twigs, and form conidia in early spring [5]. In addition to asexual reproduction, they may also produce ascospores in perithecia, which were observed on apples in dead wood and on pears in leaves [6–8].

Figure 1. Symptoms of peach anthracnose on fruit and leaves. (a–f) Various symptoms on fruit of *Prunus persica* (a–c,f) and *P. persica* var. *nucipersica* (d,e): (a,c–e) lesions on fruitlets and (b,f) lesions on mature peach fruit; (g,h) anthracnose symptoms on leaves; (i) mumified young fruit; (j) infected twig.
In the past, the taxonomy of the genus *Colletotrichum* mainly relied on host range and morphological characteristics [9]. However, these characteristics are not suitable for species-level identification since they are dependent on environmental conditions, many *Colletotrichum* species are polyphagous, and multiple species can infect the same host plant [10–13]. Molecular identification based on multilocus phylogenetic analyses or specific gene sequencing has been used for the classification and description of species concepts [3]. To date, 15 *Colletotrichum* species complexes and 22 individual species have been identified [14–16].

The causal agents of peach anthracnose were first reported as *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* [17–20]. However, the use of molecular tools for the classification of anthracnose pathogens revealed that peach anthracnose in the USA was mostly caused by *Colletotrichum nymphaeae* and *Colletotrichum fioriniae* of the *C. acutatum* species complex [21], and *Colletotrichum siamense* and *Colletotrichum fructicola* of the *C. gloeosporioides* species complex [22]. *C. nymphaeae* was also reported in Brazil on peaches [23], and *C. fioriniae*, *C. fructicola*, and *C. siamense* were identified in South Korea on peaches [24]. Peach infections by *Colletotrichum truncatum* and *Colletotrichum acutatum* are rare [25,26].

The objective of this study was to systematically identify *Colletotrichum* spp. associated with peach fruit and leaf anthracnose in China using morphological characterization and multilocus phylogenetic analyses.

2. Materials and Methods
2.1. Isolation of *Colletotrichum* spp. from Peach Samples

During 2017 and 2018, the fruit and leaves of peaches with anthracnose symptoms were collected from 14 commercial peach orchards and two nurseries (Wuhan, Hubei and Fuzhou, Fujian) in 11 provinces of China, which were dry-farmed and sprayed with fungicides for anthracnose control. Conidia on diseased tissues were dipped in a cotton swab and spread on a potato dextrose agar (PDA, 20% potato infusion, 2% glucose, and 1.5% agar, and distilled water) medium and picked up with a glass needle under a professional single spore separation microscope (Wuhan Heipu Science and Technology Ltd., Wuhan, China). If no conidia were present, leaf and fruit pieces (5 × 5 mm) at the intersection of healthy and diseased tissues were surface sterilized with a sodium hypochlorite solution (1%) for 30 s and washed three times in sterilized water, followed by 75% ethanol for 30 s, then washed three times in sterilized water again. After the tissue pieces were dried, they were placed on PDA and incubated at 25 °C with a 12 h/12 h fluorescent light/dark cycle for about seven days to produce spores. Cultures were transferred to 15% diluted oatmeal agar (0.9% oatmeal, 1.5% agar, and distilled water) plates if there was no sporulation on PDA [27]. The ex-type living culture of novel species in this study was deposited in the China Center for Type Culture Collection (CCTCC), Wuhan, China.

2.2. Morphological Characterization

Mycelial plugs (5 mm) were transferred from the edge of actively growing cultures to fresh PDA plates and incubated at 25 °C in the dark. Colony diameters were measured after three days to calculate the mycelial growth rates (mm/d). The shape and color of colonies were investigated on the sixth day. Sexual morphs of some species were produced after four weeks. The characteristics of conidiomata were observed using fluorescence stereo microscope (Leica M205 FA, Leica Microsystems Ltd., Wetzlar, Germany). Moreover, the shape and color of conidia, conidiophores, appressoria, ascomata, asci, ascospores, and setae were recorded using a light microscope (Nikon Eclipse E400, Nikon Instruments Inc., San Francisco, CA, USA), and the length and width of 30 randomly selected conidia and 30 appressoria were measured for each representative isolate. Appressoria were induced by dropping 50 µL conidial suspension (10^5 conidia/mL) on a microscope slide, which was placed inside a plate containing moistened filter papers with distilled water, and incubated at 25 °C in the dark for 24 to 48 h [28].
2.3. DNA Extraction, PCR Amplification, and Sequencing

From the 286 obtained isolates, 51 were selected for further multilocus phylogenetic analyses. They represented each geographical population, colony type, conidia morphology, and host tissue.

Fungal DNA was extracted as described previously [29]. The 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS), partial sequences of the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH), chitin synthase 1 gene (CHS-1), actin gene (ACT), beta-tubulin gene (TUB2), histone3 gene (HIS3), and calmodulin gene (CAL) were amplified and sequenced using the primer pairs described in Table S1.

The PCR conditions were 4 min at 95 °C, followed by 35 cycles of 95 °C for 30 s, annealing for 30 s at different temperatures for different genes/loci (Table S1), and 72 °C for 45 s, with a final extension at 72 °C for 7 min. DNA sequencing was performed at Tianyi Huiyuan Biotechnology Co., Ltd. (Wuhan, China) with an ABI 3730XL sequencer from Thermo Fisher Scientific (China) Co., Ltd. (Shanghai, China). The consensus sequences were assembled from forward and reverse sequences with MEGA v. 7.0 [30]. All sequences of 51 representative Colletotrichum isolates in this study were submitted to GenBank and the accession numbers are listed in Table S2.

2.4. Phylogenetic Analyses

Isolates were divided into four groups based on multilocus phylogenetic analyses, and type isolates of each species were selected and included in the analyses (Table 1). Multilocus phylogenetic analyses with concatenated ITS, GAPDH, CHS-1, HIS3, ACT, and TUB2 sequences were conducted for the C. acutatum species complex [31]; ACT, CAL, CHS-1, GAPDH, ITS, and TUB2 sequences were concatenated for the analysis of the C. gloeosporioides species complex [32]; the combined ITS, GAPDH, CHS-1, HIS3, ACT, TUB2, and CAL sequences were used to analyze the C. boninense species complex [33]; and the ITS, GAPDH, CHS-1, ACT, and TUB2 sequences were applied for remaining species [34]. Multiple sequences were aligned and combined using MAFFT v.7 [35] and MEGA v.7.0 [30].

Bayesian inference (BI) was used to construct phylogenetic trees in MrBayes v.3.2.2 [36]. Best-fit models of nucleotide substitution were selected using MrModeltest v.2.3 [37] based on the corrected Akaike information criterion (AIC) (Tables 2–5). BI analyses were launched with two MCMC chains that were run for $1 \times 10^6$ generations (C. acutatum species complex and C. boninense species complex) [31,33], and trees sampled every 100 generations; or run $1 \times 10^7$ generations (C. gloeosporioides species complex, and remaining species) [8,34], and trees sampled every 1000 generations. The calculation of BI analyses was stopped when the average standard deviation of split frequencies fell below 0.01. On this basis, the first 25% of generations were discarded as burn-in. Maximum parsimony (MP) analyses were implemented by using Phylogenetic Analysis Using Parsimony (PAUP*) v.4.0b10 [38]. Goodness of fit values including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for the bootstrap analyses (Tables 2–5). Phylogenetic trees were generated using the heuristic search option with Tree Bisection Reconnection (TBR) branch swapping and 1000 random sequence additions, with all characters equally weighted and alignment gaps treated as missing data. Maximum likelihood (ML) analyses were carried out by using the CIPRES Science Gateway v.3.3 (www.phylo.org, accessed on 29 December 2021), while RAxML-HPC BlackBox was selected with default parameters. Phylogenetic trees were visualized in FigTree v.1.4.2 [39]. TreeBASE was used to store the concatenated multilocus alignments (submission number: 29227).
Table 1. Strains used for the phylogenetic analysis of *Colletotrichum* spp. and other species with details about host, location, and GenBank accession numbers.

| Species            | Culture a   | Host                        | Location    | GenBank Accession Number |
|--------------------|-------------|-----------------------------|-------------|--------------------------|
| C. acerbum         | CBS 128530 *| *Malus domestica*           | New Zealand | JQ948459 JQ948790 JQ949120 JQ949780 JQ949450 JQ950110 - |
| C. acutatum        | CBS 112996 *| *Carica papaya*             | Australia   | JQ005776 JQ048677 JQ005797 JQ005839 JQ005818 JQ005860 - |
| C. aenigma         | ICMP 18608 *| *Persea americana*          | Israel      | JX010244 JX010044 JX009774 JX009443 - JX010389 JX009683 |
| C. aescynomenes    | ICMP 17673 *| *Aescynomone virginica*     | USA         | JX010176 JX009930 JX009799 JX009483 - JX010392 JX009721 |
| C. agaves          | CBS 118190  | *Agnave striate*            | Mexico      | DQ286221 - - - - - - |
| C. alatae          | ICMP 17919 *| *Dioscorea alata*           | India       | JX010190 JX009990 JX009837 JX009471 - JX010383 JX009738 |
| C. aliatum         | ICMP 12071 *| *Malus domestica*           | New Zealand | JX010251 JX010028 JX009882 JX009572 - JX010411 JX009654 |
| C. annellatum      | CBS 129826 *| *Hevea brasiliensis*        | Colombia    | JQ005222 JQ005309 JQ005396 JQ005570 JQ005483 JQ005656 JQ005743 |
| C. aotearoa        | ICMP 18537 | *Coprosma sp.*              | New Zealand | JX010205 JX010005 JX009853 JX009564 - JX010420 JX009611 |
| C. arecicola       | CGMCC 3.19667* | *Areca catechu*           | China       | MK914635 MK935455 MK935541 MK935374 - MK935498 - |
| C. artocarpica     | MFLJCC 18-1167 | *Artocarpus heterophyllus* | Thailand    | MN145999 MN435568 MN435569 MN435570 - MN435567 - |
| C. arxii           | CBS 132511 | *Paphepiedus sp.*           | Germany     | KF687716 KF687843 KF687800 KF687802 - KF687881 - |
| C. asiaticum       | ICMP 18580 | *Coffee arabica*            | Thailand    | FJ972612 JX010053 JX009867 JX009584 - JX010406 FJ97506 |
| C. australis       | CBS 116478 *| *Trachycarpus fortunei*     | South Africa | JQ48455 JQ948786 JQ49116 JQ49977 JQ49144 JQ950106 - |
| C. bambusicola     | CFCC 54250 *| *Phyllostachys edulis*      | China       | MT199632 MT192848 MT192871 MT188638 - MT192817 - |
| C. becqueri        | CBS 128527 *| *Brachyglossis repanda*    | New Zealand | JX005171 JQ005258 JQ005345 JQ005519 JQ005432 JQ005605 JQ005692 |
| C. boninense       | CBS 123755 | *Crinum asiaticum*          | Japan       | JQ005153 JQ005240 JQ005327 JQ005501 JQ005414 JQ005588 JQ005674 |
| C. brasiliense     | CBS 128501 *| *Passiflora edulis*         | Brazil      | JQ005235 JQ005322 JQ005409 JQ005583 JQ005496 JQ005669 JQ005756 |
| C. brassicicola    | CBS 101059 *| *Brassica olerace var.*     | New Zealand | JQ005172 JQ005259 JQ005346 JQ005520 JQ005433 JQ005606 JQ005693 |
| C. brisbanense     | CBS 292.67 *| *Capsicum annuum*           | Australia   | JQ498291 JQ498621 JQ498952 JQ49612 JQ499282 JQ49942 - |
| C. cairnsense      | CBS 140847 *| *Capsicum annuum*           | Australia   | KU923672 KU923704 KU923710 KU923722 KU923688 - |
| C. camelliae-japonica | CGMCC 3.18118 | *Camellia japonica*       | Japan       | KX853165 KX893584 - KX893576 - KX893580 - |
| Species                | Culture a | Host                   | Location  | GenBank Accession Number   |
|------------------------|-----------|------------------------|-----------|----------------------------|
|                        |           |                        | ITS       | GAPDH | CHS-1 | ACT | HIS3 | TUB2 | CAL |
| C. chlorophyti         | IMI 103806 * | Chlorophyllum sp.      | India     | GU227894 GU228286 GU228384 GU227992 | - | GU228188 | - |
| C. chrysanthemi        | IMI 364540 | Chrysanthemum coronarium | China     | JQ948273 JQ948603 JQ948934 JQ949594 | JQ949264 | JQ949924 | - |
| C. ciggaro             | ICMP 18539 * | Olea europaea         | Australia | JX010230 JX009966 JX009800 JX009523 | JX010434 | JX009635 | - |
|                        | CBS 237.49 * | Hypericum perforatum | Germany   | JX010238 JX010042 JX009840 JX009450 | JX010432 | JX009636 | - |
| C. citricola           | CBS 134228 * | Citrus unshiu        | China     | KC293576 KC293736 - | KC293616 | - | KC293656 KC293696 |
| C. citrus-medicae      | HGUP 1554 *, GUCC 1554 | Citrus medica | China     | MN959910 MT006331 MT006328 MT006326 MT006335 | - | - |
|                        | GUCC 1555 | Citrus medica         | China     | MN959911 MT006332 MT006329 MT006326 MT006335 | - | - |
|                        | GUCC 1556 | Citrus medica         | China     | MN959912 MT006333 MT006330 MT006327 MT006336 | - | - |
| C. clademia             | ICMP 18658 * | Clidemia hirta       | USA       | JX010265 JX009989 JX009877 JX009537 | JX010438 | JX009645 | - |
| C. colombiense          | CBS 129818 * | Passiflora edulis   | Colombia  | JQ005174 JQ005261 JQ005348 JQ005522 JQ005435 JQ005648 JQ005695 | JQ005608 JQ005695 |
| C. constrictum         | CBS 128504 * | Citrus limon         | New Zealand | JQ005238 JQ005325 JQ005412 JQ005586 JQ005658 | JQ005672 | JQ005759 | - |
| C. cordylinicola       | ICMP 18579 * | Cordyline fruticosa | Thailand  | JX010226 JX009975 JX009864 HM470235 | - | JX010440 HM470238 |
| C. curcumae            | IMI 288937 * | Curcuma longa       | India     | GU227893 GU228285 GU228383 GU227991 | GU228187 | - |
| C. cuscutae            | IMI 304802 * | Cuscuta sp.         | Dominica  | JQ948195 JQ948525 JQ948856 JQ949516 JQ949846 | - |
| C. cymbidicola         | IMI 347923 * | Cymbidium sp.       | Australia | JQ005166 JQ005253 JQ005340 JQ005514 JQ005427 JQ005600 JQ005677 JQ005757 |
| C. dacyrcarpi          | CBS 130241 | Dacrycarpus dacrydii | New Zealand | JQ005236 JQ005323 JQ005410 JQ005584 JQ005497 JQ005670 JQ005757 |
| C. dracaeophilum       | CBS 118199 | Dracaena sp.         | China     | JX519222 JX546707 JX519230 JX519238 | - | JX519247 | - |
| C. criobotryae         | BCRC      | Eriobotrya japonica  | China     | MF772487 MF795423 MN191653 MN191668 MN191668 | MF795428 | - |
| C. ephorbiae           | CBS 134725 * | Euphorbia sp.       | South Africa | KF777146 KF777131 KF777128 KF777125 | - | KF777247 | - |
| C. floriniae           | CBS 128517 * | Fornia externa      | USA       | JQ498292 JQ498622 JQ498953 JQ498613 JQ499283 JQ499943 |
|                        | IMI 324296 | Malus pumila        | USA       | JQ498301 JQ498631 JQ498962 JQ498622 JQ499292 JQ499952 | - |
|                        | CBS 126526 | Prunus sp.          | Netherlands | JQ498232 JQ498653 JQ498954 JQ499644 JQ499314 JQ499974 | - |
|                        | CBS 124958 | Pyrus sp.           | USA       | JQ498306 JQ498636 JQ498967 JQ498627 JQ499297 JQ499957 | - |
|                        | CBS 119292 | Vaccinium sp.       | New Zealand | JQ498313 JQ498643 JQ498974 JQ499634 JQ499304 JQ499964 | - |
Table 1. Cont.

| Species          | Culture a | Host         | Location    | GenBank Accession Number |
|------------------|-----------|--------------|-------------|--------------------------|
|                  |           |              |             | ITS | GAPDH | CHS-1 | ACT | HIS3 | TUB2 | CAL |
| ICKb31           | Prunus persica | South Korea  | LC516639    | LC516653 | LC516660 | - | - | - | LC516646 | - |
| ICKb36           | Prunus persica | South Korea  | LC516640    | LC516654 | LC516661 | - | - | - | LC516647 | - |
| ICKb47           | Prunus persica | South Korea  | LC516641    | LC516655 | LC516662 | - | - | - | LC516648 | - |
| C.2.4.2          | Prunus persica | USA         | KX006691    | KX006694 | -  | - | - | - | KX066888 | - |
| CaEY12_1         | Prunus persica | USA         | KX006693    | KX006696 | -  | - | - | - | KX066909 | - |
| **C. fructicola** |           |              |             |     |     |     |     |     |     |     |
| ICMP 18581 *     | Coffea arabica | Thailand    | JX010165    | JX010033 | JX009866 | FJ907426 | - | JX010405 | - |
| ICMP 18613 *     | Limonium sinuatum | Israel    | JX010167    | JX009998 | JX009722 | JX009491 | - | JX010388 | JX09675 |
| ICMP 18581 *     | Coffea arabica | Thailand    | JX010165    | JX010033 | JX009866 | FJ907426 | - | JX010405 | FJ917508 |
| ICMP 18727       | Fragaria × ananassa | USA    | JX010179    | JX010035 | JX009812 | JX009565 | - | JX010394 | JX09682 |
| CBS 125397 *     | Tetrathyris panamensis | Panama    | JX010132    | JX010032 | JX009745 | JX009581 | - | JX010409 | JX09674 |
| CBS 238.49 *     | Ficus edulis | Germany     | JX010181    | JX009923 | JX009839 | JX009495 | - | JX010400 | JX09671 |
| ICKb18           | Prunus persica | South Korea  | LC516635    | LC516649 | LC516656 | - | - | - | LC516642 | LC516663 |
| ICKb132          | Prunus persica | South Korea  | LC516636    | LC516650 | LC516657 | - | - | - | LC516643 | LC516664 |
| RR12-3           | Prunus persica | USA         | -           | -         | -       | - | - | - | KM245092 | KJ769239 |
| SE12-1           | Prunus persica | USA         | -           | KJ769247 | -       | - | - | - | KJ769237 | KJ769237 |
| **C. fusiforme**  |           |              |             |     |     |     |     |     |     |     |
| MFLUCC 12-0437 * |         | Thailand     | KT290266    | KT290255 | KT290253 | KT290251 | - | KT290256 | - |
| C. gigasporum    | CBS 133266 * | Centella asiatica | Madagascar  | KF687715 | KF687822 | KF687761 | - | - | KF68766 | - |
| C. gloeosporioides| CBS 112999 * | Citrus sinensis | Italy       | JQ005152 | JQ005239 | JQ005326 | JQ005500 | JQ005413 | JQ005587 | - |
| ICMP 17821 *     | Citrus sinensis | Italy    | JX010152    | JX010056 | JX009818 | JX009531 | - | JX010445 | JX09731 |
| CBS 796.72       | Aeschynomene virginica | USA    | JQ948407    | JQ948738 | JQ949068 | JQ949728 | - | JQ950058 | - |
| CBS 133.44 *     | Clarkia hybrid | Denmark     | JQ948402    | JQ948733 | JQ949063 | JQ949723 | JQ949393 | - | JQ950053 | - |
| IMI 351248       | Camptothecus sp. | UK         | JQ948433    | JQ948764 | JQ949094 | JQ949754 | JQ94924 | - | JQ950084 | - |
| C. guangxiense   | CFCC 54251 * | Phyllostachys edulis | China     | MT199633 | MT192834 | MT192861 | MT188628 | - | MT192805 | - |
| C. hippocastri   | Dipsacus vitatum | China      | JQ005231    | JQ005318 | JQ005405 | JQ005579 | JQ005492 | JQ005665 | JQ005752 |
| C. horii         | ICMP 10492 * | Diospyros kaki | Japan       | GQ329690 | GQ329681 | JX009752 | JX009438 | - | JX010450 | JX09604 |
| C. indonesiense  | CBS 127551 * | Eucalyptus sp. | Indonesia   | JQ948228 | JQ948618 | JQ948949 | JQ949609 | JQ949279 | JQ949939 | - |
| C. javanense     | CBS 144963 * | Capsicum annuum | Indonesia   | MH846576 | MH846572 | MH846573 | MH846575 | - | MH846574 | - |
Table 1. Cont.

| Species            | Culture a | Host                     | Location   | GenBank Accession Number |
|--------------------|-----------|--------------------------|------------|--------------------------|
|                    |           |                          | ITS | GAPDH | CHS-1 | ACT | HIS3 | TUB2 | CAL |
| *C. jishouense*    | GZU_HJ2_G2 | *Nothapodytes pittosporoides* | China | MH482931 | MH681657 | -   | MH708134 | -   | MH727472 | -   |
| *C. johnstonii*    | CBS 128532 | *Solanum lycopersicum*   | New Zealand | JQ948444 | JQ948775 | JQ949105 | JQ949765 | JQ949435 | JQ950095 | -   |
| *C. kahawae*       | IMI 319418* | *Coffee arabica*       | Kenya | JX010231 | JX010012 | JX009813 | JX009452 | -   | JX010444 | -   |
| *C. karsti*        | CBS 128524 | *Citrus lanatus*        | New Zealand | JQ005195 | JQ005282 | JQ005369 | JQ005543 | JQ005456 | JQ005456 | JQ005629 | JQ005716 |
| *C. kahawae*       | CBS 129824 | *Musa AAA*              | Colombia | JQ005215 | JQ005302 | JQ005389 | JQ005563 | JQ005476 | JQ005449 | JQ005649 | JQ005736 |
| *C. kahawae*       | CBS 128552 | *Synsepalum dulcificum* | Taiwan | JQ005188 | JQ005275 | JQ005362 | JQ005536 | JQ005449 | JQ005622 | JQ005709 |
| *C. johnstonii*    | CBS 128532 | *Hevea brasiliensis*    | India | JQ948289 | JQ948619 | JQ948950 | JQ949610 | JQ949280 | JQ949940 | -   |
| *C. ledebouriae*   | CBS 141284* | *Ledebouria floridunda* | South Africa | KX228254 | -   | -   | -   | -   | -   | -   |
| *C. liasoningense* | CGMCC 3.17616* | *Capsicum sp.*         | China | KP890104 | KP890135 | KP890127 | KP890097 | -   | KP890111 | -   |
| *C. limetickola*   | CBS 114.14* | *Citrus aurantifolia*   | USA | JQ948193 | JQ948523 | JQ948854 | JQ949514 | JQ949184 | JQ949844 | -   |
| *C. lindeimuthianum* | CBS 144.31* | *Phaeolus vulgaris*     | Germany | JQ005779 | JX546712 | JQ005800 | JQ005842 | -   | JQ005863 | -   |
| *C. magnisporum*   | CBS 398.84* | unknown               | unknown | KF687718 | KF687842 | KF687782 | KF687803 | -   | KF687882 | -   |
| *C. magnus*        | CBS 519.97* | *Citrus lanatus*        | USA | MG600769 | MG600829 | MG600875 | MG600973 | -   | MG601036 | -   |
| *C. makassarensis* | CBS 143664* | *Capsicum annuum*       | Indonesia | MH728812 | MH728820 | MH780580 | MH781480 | -   | MH846563 | -   |
| *C. musae*         | CBS 116870* | *Musa sp.*              | USA | JX010146 | JX010050 | JX009896 | JX009433 | -   | HQ396280 | JX009742 |
| *C. neosansevieriae* | CBS 139918* | *Sansevieria trifasciata* | South Africa | KR476747 | KR476791 | -   | KR476790 | -   | KR476797 | -   |
| *C. novae-zelandiae* | CBS 128505* | *Capsicum annuum*       | New Zealand | JQ005228 | JQ005315 | JQ005402 | JQ005576 | JQ005489 | JQ005662 | JQ005749 |
| *C. nupharicola*   | ICMP 18187* | *Nuphar lutea subsp.polysepala* | USA | JX010187 | JX009972 | JX009835 | JX009437 | -   | JX010398 | JX009663 |
| *C. nympheae*      | CBS 515.78* | *Nymphaea alba*         | Netherlands | JQ948197 | JQ948527 | JQ948858 | JQ949518 | JQ949188 | JQ949848 | -   |
| *C. nympheae*      | CBS 130.80 | *Anemone sp.*           | Italy | JQ948226 | JQ948356 | JQ948887 | JQ949547 | JQ949217 | JQ949877 | -   |
| *C. nympheae*      | IMI 360386 | *Pelargonium graveolens* | India | JQ948206 | JQ948536 | JQ948867 | JQ949271 | JQ949197 | JQ949837 | -   |
| *C. nympheae*      | CBS 125973 | *Fragaria × ananassa*   | UK | JQ948232 | JQ948562 | JQ948893 | JQ949553 | JQ949223 | JQ949883 | -   |
| PrpCnSC13-01       | Prunus persica | USA | KX066092 | KX066095 | -   | -   | -   | KX066089 | -   |
| PrpCnSC13-02       | Prunus persica | Brazil | MK761066 | MK770424 | MK770421 | -   | -   | MK770427 | -   |
| PrpCnSC13-02       | Prunus persica | Brazil | MK765508 | MK770425 | MK770422 | -   | -   | MK770428 | -   |
| Species | Culture | Host | Location | GenBank Accession Number |
|---------|---------|------|----------|-------------------------|
|         |         |      |          | ITS         | GAPDH      | CHS-1      | ACT       | HIS3      | TUB2      | CAL       |
| C. oncidii | CBS 129828 | Oncidium sp. | Germany | JQ005169 | JQ005256 | JQ005343 | JQ005517 | JQ005430 | JQ005603 | JQ005690 |
| C. orbiculare | CBS 570.97 | Cucumis sativus | Europe | KF178466 | KF178490 | KF178515 | KF178563 | -            | KF178587 | -            |
| C. orchidearum | CBS 135131 | Dendrobium nobile | Netherlands | MG600738 | MG600800 | MG600855 | MG600944 | -            | MG601005 | -            |
| C. orchidophilum | CBS 632.80 | Dendrobium sp. | USA | JQ948151 | JQ948481 | JQ948812 | JQ949472 | JQ949142 | JQ949802 | -            |
| C. parsonsiæ | CBS 128525 | Parsonsiæ capsularis | New Zealand | JQ005233 | JQ005320 | JQ005407 | JQ005581 | JQ005494 | JQ005667 | JQ005754 |
| C. paxtonii | IMI 165753 | Musa sp. | Saint Lucia | JQ948285 | JQ948615 | JQ948946 | JQ949606 | JQ949276 | JQ949936 | -            |
| C. petchii | CBS 378.94 | Dracaena marginata | Italy | JQ005223 | JQ005310 | JQ005397 | JQ005581 | JQ005494 | JQ005667 | JQ005744 |
| C. phormii | CBS 118194 | Phormium sp. | Germany | JQ948446 | JQ948777 | JQ949107 | JQ949767 | JQ949437 | JQ950097 | -            |
| C. phyllanthi | CBS 175.67 | Phyllanthus acidus | India | JQ005221 | JQ005308 | JQ005395 | JQ005569 | JQ005482 | JQ005655 | JQ005742 |
| C. piperis | IMI 57197 | Piper nigrum | Malaysia | MG600760 | MG600820 | MG600867 | MG600964 | -            | MG601027 | -            |
| C. pseudomajus | CBS 571.88 | Camellia sinensis | China | KF687722 | KF687826 | KF687799 | KF687801 | -            | KF687883 | -            |
| C. psidii | CBS 145.29 | Psidium sp. | Italy | JX010219 | JX009967 | JX009901 | JX009515 | -            | JX010443 | JX009743 |
| C. pyricola | CBS 128531 | Pyrus communis | New Zealand | JQ948445 | JQ948776 | JQ949106 | JQ949766 | JQ949436 | JQ950096 | -            |
| C. pyrifoliale | CGMCC 3.18902 | Pyrus pyrifolia | China | MG748078 | MG747996 | MG747914 | MG747768 | -            | MG748158 | -            |
| C. queenslandicum | ICMP 1778 | Carica papaya | Australia | JX010276 | JX009934 | JX009899 | JX009447 | -            | JX010414 | JX096911 |
| C. radicans | CBS 529.93 | unknown | Costa Rica | KF687719 | KF687825 | KF687762 | KF687875 | -            | KF687869 | -            |
| C. salicis | CBS 607.94 | Salix sp. | Netherlands | JQ948460 | JQ948791 | JQ949121 | JQ949781 | JQ949451 | JQ950111 | -            |
| C. salsoleae | ICMP 19051 | Salsola tragus | Hungary | JX010242 | JX009916 | JX009863 | JX009562 | -            | JX010403 | JX096966 |
| C. sansevieriae | MAFF 239721 | Sansevieria trifasciata | Japan | AB212991 | -            | -            | -            | -            | -            | -            |
| C. scovillei | CBS 126529 | Capsicum sp. | Indonesia | JQ948267 | JQ948597 | JQ948928 | JQ949588 | JQ949258 | JQ949918 | -            |
| C. siamense | ICMP 18578 | Coffea arabica | Thailand | JX010171 | JX009924 | JX009865 | FJ907423 | -            | JX010404 | FJ917505 |
| C. siamense (syn. C. hymenocallisidis) | CBS 125378 | * | China | JX010278 | JX010019 | GQ856730 | GQ856775 | -            | JX010410 | JX097079 |
| C. siamense (syn. C. jasmini-sambac) | CBS 130420 | * | Vietnam | HM131511 | HM131497 | JX009895 | HM131507 | -            | JX010415 | JX097131 |
| ICKb21 | Prunus persica | South Korea | LC516637 | LC516651 | LC516658 | -            | -            | LC516644 | LC516665 |
| ICKb23 | Prunus persica | South Korea | LC516638 | LC516652 | LC516659 | -            | -            | LC516645 | LC516666 |
| OD12-1 | Prunus persica | USA | -            | KJ769240 | -            | -            | -            | KM245089 | KJ769234 |
Table 1. Cont.

| Species | Culture a | Host | Location | GenBank Accession Number |
|---------|-----------|------|----------|--------------------------|
|         |           |      |          |                          |
| EY12-1  | Prunus persica | USA | -        | KJ769246 -        |
| C. simondsii | CBS 122122 * | Carica papaya | Australia | JQ948276 JQ948606 JQ948937 JQ949597 JQ949267 JQ949927 - |
| C. sloanei | IMI 364297 * | Theobroma cacao | Malaysia | JQ948287 JQ948617 JQ948948 JQ949608 JQ949278 JQ949938 - |
| C. sojae | ATCC 62257 * | Glycine max | USA | MG600749 MG600810 MG600860 MG600954 - MG601016 - |
| C. sydowi | CBS 135819 | Sambucus sp. | China | KY263783 KY263785 KY263787 KY263791 - KY263793 - |
| C. tainanense | CBS 143666 * | Capsicum annuum | Taiwan | MH728818 MH728823 MH805845 MH781475 - MH846558 - |
| C. theobromicola | CBS 124945 * | Theobroma cacao | Panama | JX010294 JX010006 JX009869 JX09444 JX010447 JX009591 |
| C. ti | ICMP 4832 * | Cordyline sp. | New Zealand | JX010269 JX009952 JX009898 JX009520 - JX010442 JX09649 |
| C. tongrenense | GZU_TRJ1-37 | Notaphytopites pittosporoides | China | MH482933 MH705332 - MH717074 - MH729805 - |
| C. torulosum | CBS 128544 * | Solanum melongena | New Zealand | JQ005164 JQ005251 JQ005338 JQ005512 JQ005425 JQ005598 JQ005685 |
| C. trichellum | CBS 217.64 * | Hedera helix | UK | GU227812 GU228204 GU228302 GU227910 - GU228106 - |
| C. tropicale | CBS 124949 * | Theobroma cacao | Panama | JX010264 JX010007 JX009870 JX009489 JX100407 JX09719 |
| C. truncatum | CBS 151.35 * | Phaseolus lunatus | USA | GU227862 GU228254 GU228352 GU227960 - GU228156 - |
| C. vietnamense | CBS 125478 * | Coffea sp. | Vietnam | KF687721 KF687832 KF687769 KF687792 - KF687877 - |
| C. walleri | CBS 125472 * | Coffea sp. | Vietnam | JQ948275 JQ948605 JQ948936 JQ949596 JQ949266 JQ949926 - |
| C. wanningense | CGMCC 3.18936 * | Hevea brasiliensis | China | MG830462 MG830318 MG830302 MG830270 - MG830286 - |
| C. waxiens | CGMCC 3.17894 * | Camellia sinensis | China | KU251591 KU252045 KU251939 KU251672 - KU251833 - |
| C. xanthorrhoeae | ICMP 17903 * | Xanthorrhoea preissii | Australia | JX010261 JX009927 JX009823 JX009478 - JX010448 JX09653 |
| C. yunnanense | CBS 132135 * | Buxus sp. | China | JX056804 JX546706 JX519231 JX519239 - JX519248 - |
| Monilochaetes infuscans | CBS 869.96 * | Ipomoea batatas | South Africa | JQ005780 JX546612 JQ005801 JQ005843 - JQ005864 - |

a CBS: Culture collection of the Centraalbureau voor Schimmelcultures; ICMP: International Collection of Microorganisms from Plants, Auckland, New Zealand; CGMCC: China General Microbiological Culture Collection; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; BCRC: Bioresource Collection and Research Center, Hsinchu, Taiwan; MFLU: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; ATCC: American Type Culture Collection. * = Ex-holotype or ex-epitype cultures.
Table 2. Comparison of alignment properties in parsimony analyses of gene/locus and nucleotide substitution models used in phylogenetic analyses of C. acutatum species complex.

| Gene/Locus | ITS     | GAPDH   | CHS-1   | HIS3    | ACT    | TUB2    | Combined |
|------------|---------|---------|---------|---------|--------|---------|----------|
| No. of taxa| 72      | 72      | 68      | 60      | 63     | 72      | 72       |
| Aligned length (with gaps) | 546 | 265 | 282 | 387 | 248 | 492 | 2240 |
| Invariable characters | 501 | 152 | 244 | 289 | 170 | 374 | 1750 |
| Uninformative variable characters | 26 | 56 | 13 | 32 | 30 | 60 | 217 |
| Phylogenetically informative characters | 19 | 57 | 25 | 66 | 48 | 58 | 273 |
| Tree length (TL) | 59 | 176 | 64 | 190 | 117 | 165 | 872 |
| Consistency index (CI) | 0.85 | 0.80 | 0.73 | 0.66 | 0.75 | 0.79 | 0.71 |
| Retention index (RI) | 0.97 | 0.95 | 0.94 | 0.93 | 0.94 | 0.94 | 0.93 |
| Rescaled consistency index (RC) | 0.82 | 0.76 | 0.69 | 0.61 | 0.71 | 0.75 | 0.65 |
| Homoplasy index (HI) | 0.15 | 0.20 | 0.27 | 0.34 | 0.25 | 0.21 | 0.30 |
| Nucleotide substitution model | HKY + I | HKY + G | K80 + I | GTR + I + G | GTR + G | GTR + G | GTR + I + G |

Table 3. Comparison of alignment properties in parsimony analyses of gene/locus and nucleotide substitution models used in phylogenetic analyses of C. gloeosporioides species complex.

| Gene/Locus | ACT | CAL | CHS-1 | GAPDH | ITS | TUB2 | Combined |
|------------|-----|-----|-------|-------|-----|------|----------|
| No. of taxa | 54  | 58  | 58    | 62    | 58  | 58   | 61       |
| Aligned length (with gaps) | 314 | 744 | 300   | 307   | 614 | 735 | 3034 |
| Invariable characters | 232 | 520 | 239   | 154   | 555 | 489 | 2209 |
| Uninformative variable characters | 54  | 139 | 22    | 77    | 36  | 156 | 484 |
| Phylogenetically informative characters | 28  | 85  | 39    | 76    | 23  | 90  | 341 |
| Tree length (TL) | 115 | 324 | 102   | 264   | 78  | 349 | 1303 |
| Consistency index (CI) | 0.84 | 0.83 | 0.69 | 0.75 | 0.81 | 0.83 | 0.76 |
| Retention index (RI) | 0.85 | 0.92 | 0.84 | 0.84 | 0.87 | 0.87 | 0.84 |
| Rescaled consistency index (RC) | 0.71 | 0.76 | 0.58 | 0.63 | 0.70 | 0.72 | 0.63 |
| Homoplasy index (HI) | 0.17 | 0.17 | 0.31 | 0.25 | 0.19 | 0.17 | 0.24 |
| Nucleotide substitution model | HKY + G | GTR + G | K80 + G | HKY + I | SYM + I + G | HKY + I | GTR + I + G |

New species and their most closely related neighbors were analyzed using the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) model by performing a pairwise homoplasy index (PHI) test [40]. The PHI test was carried out on SplitsTree v.4.14.6 [41,42] using concatenated sequences (ITS, GAPDH, CHS-1, ACT, and HIS3). The result of pairwise homoplasy index below a 0.05 threshold (Φw < 0.05) indicated the presence of significant recombination in the dataset. The relationship between closely related species was visualized by constructing a splits graph. In addition, the results of relationships between closely related species were visualized by constructing EqualAngle splits graphs, using both LogDet character transformation and split decomposition distances options.
### Table 4. Comparison of alignment properties in parsimony analyses of gene/locus and nucleotide substitution models used in phylogenetic analyses of C. boninense species complex.

| Gene/Locus | ITS | GAPDH | CHS-1 | HIS3 | ACT | TUB2 | CAL | Combined |
|------------|-----|-------|-------|------|-----|------|-----|----------|
| No. of taxa| 25  | 25    | 23    | 23   | 25  | 25   | 24  | 25       |
| Aligned length (with gaps) | 553 | 286   | 393   | 276  | 302 | 502  | 249 | 2763     |
| Invariable characters | 489 | 120   | 224   | 295  | 174 | 348  | 259 | 1932     |
| Phylogenetically informative characters | 40  | 82    | 25    | 28   | 53  | 75   | 103 | 408      |
| Tree length (TL) | 24  | 84    | 31    | 70   | 49  | 79   | 87  | 423      |
| Consistency index (CI) | 0.86| 0.80  | 0.76  | 0.66 | 0.82| 0.75 | 0.80| 0.76     |
| Retention index (RI) | 0.88| 0.79  | 0.79  | 0.79 | 0.83| 0.75 | 0.85| 0.79     |
| Rescaled consistency index (RC) | 0.75| 0.64  | 0.60  | 0.52 | 0.68| 0.56 | 0.70| 0.60     |
| Homoplasy index (HI) | 0.14| 0.20  | 0.24  | 0.34 | 0.18| 0.25 | 0.18| 0.24     |
| Nucleotide substitution model | SYM + I + G | HKY + I | K80 + G | GTR + I + G | GTR + G | HKY + I | HKY + G | GTR + I + G |

### Table 5. Comparison of alignment properties in parsimony analyses of gene/locus and nucleotide substitution models used in phylogenetic analyses of C. folicola and other taxa.

| Gene/Locus | ITS | GAPDH | CHS-1 | ACT | TUB2 | combined |
|------------|-----|-------|-------|-----|------|----------|
| No. of taxa| 50  | 47    | 44    | 47  | 44   | 50       |
| Aligned length (with gaps) | 571 | 321   | 265   | 279 | 529  | 1981     |
| Invariable characters | 367 | 63    | 163   | 102 | 223  | 934      |
| Phylogenetically informative characters | 53  | 21    | 20    | 39  | 50   | 183      |
| Tree length (TL) | 151 | 257   | 82    | 138 | 256  | 864      |
| Consistency index (CI) | 630 | 1312  | 389   | 671 | 1300 | 4405     |
| Retention index (RI) | 0.51| 0.44  | 0.41  | 0.48| 0.44 | 0.44     |
| Rescaled consistency index (RC) | 0.76| 0.68  | 0.66  | 0.71| 0.67 | 0.68     |
| Homoplasy index (HI) | 0.39| 0.30  | 0.27  | 0.34| 0.30 | 0.30     |
| Nucleotide substitution model | GTR + I + G | HKY + I + G | GTR + I + G | HKY + I + G | HKY + I + G | GTR + I + G |

#### 2.5. Pathogenicity Test

Two to five isolates of each Colletotrichum sp. were used in pathogenicity tests on detached fruit and leaves. The experimental varieties for fruit and leaf inoculations were “Xiaohong” and “Xiahui No. 5”, respectively. Commercially mature fruit (still firm but with no green background color) and asymptomatic, fully developed leaves with short twigs (1–2 cm) were washed with soap and water, and surface sterilized in 1% sodium hypochlorite for 2 min and 30 s, respectively, then rinsed with sterile water and air-dried on sterile paper. Fruit was stabbed with sterilized toothpicks to produce wounds of about 5 mm deep, while leaves were punctured with sterile, medical needles. For inoculation, a 10-µL droplet of conidia suspension (1.0–2.0 × 10^5 conidia/mL) was dropped on each
wounded site, and control fruit or leaves received sterile water without conidia. Each fruit and leaf had two inoculation sites. Three fruits and three leaves were used for each isolate. Inoculated fruit and leaves were placed in a plastic tray onto 30 mm diameter plastic rings for stability. The bottom of the tray (65 cm × 40 cm × 15 cm, 24 peaches or leaves per tray) contained wet paper towels and the top was sealed with plastic film to maintain humidity. Peaches and leaves were incubated at 25 °C for six days. Pathogenicity was evaluated by the infection rates and lesion diameters. The infection rates were calculated by the formula (\(\%\)) = (infected inoculation sites/all inoculation sites) × 100%. The lesion size was determined as the mean of two perpendicular diameters. The experiment was performed twice.

The fungus was re-isolated from the resulting lesions and identified as described above, thus fulfilling Koch’s postulates.

3. Results

From 2017 to 2018, a total of 286 *Colletotrichum* isolates were obtained from 11 provinces in China (Table 6; Figure 2a); 33 isolates were from leaves and 253 isolates were from fruit (Table 6). Although we tried to collect samples in Gansu and Shanxi provinces in northern China, no symptomatic leaves or fruit were found. *C. nymphaeae* was the most widespread and most prevalent species (Figure 2b,c), with presence in Hubei, Guizhou, Guangxi, Fujian, and Sichuan provinces. *C. fioriniae* was found in three centrally located provinces (Zhejiang, Guizhou, and Jiangxi). *C. siamense* was only found in the northernmost orchards of the collection area in Shandong and Hebei provinces, while *C. fructicola* was only found in the southernmost provinces of the collection area of Guangdong and Guizhou provinces. *C. folicola*, *C. godetiae*, and *C. karsti* were only found in Yunnan province in the westernmost border of the collection area (Table 6; Figure 2a).

Table 6. A list of all *Colletotrichum* isolates collected from peaches in China based on preliminary identification.

| Species      | Location                  | Host                          | Number of Isolates | Date           | Daily Mean Temperature (°C) |
|--------------|---------------------------|-------------------------------|--------------------|----------------|-----------------------------|
| *C. fioriniae* | Lishui, Zhejiang          | Juicy peach, Yanhong, fruit   | 17                 | 14 September 2017 | 29                          |
|              | Tongren, Guizhou          | Juicy peach, fruit            | 14                 | 8 August 2018   | 29                          |
|              | Jian, Jiangxi             | Yellow peach, fruit           | 6                  | 21 August 2018  | 31                          |
| *C. folicola* | Honghe, Yunnan            | Winter peach, Hongxue, leaf   | 2                  | 17 August 2017  | 26                          |
| *C. fructicola* | Heyuan, Guangdong    | Juicy peach, fruit           | 19                 | 28 June 2017    | 29                          |
|              | Shaoguan, Guangdong       | Juicy peach, Yingzui, fruit  | 10                 | 3 August 2018   | 30                          |
|              | Tongren, Guizhou          | Juicy peach, fruit           | 10                 | 8 August 2018   | 29                          |
| *C. godetiae* | Honghe, Yunnan            | Winter peach, Hongxue, leaf   | 15                 | 17 August 2017  | 26                          |
| *C. karsti*  | Honghe, Yunnan            | Winter peach, Hongxue, leaf   | 3                  | 17 August 2017  | 26                          |
| *C. nymphaeae* | Yichang, Hubei            | Yellow peach, NJC83, fruit   | 11                 | 30 April 2017   | 19                          |
|              | Jingmen, Hubei            | Yellow peach, NJC83, fruit   | 14                 | 25 April 2017   | 18                          |
|              | Jingmen, Hubei            | Juicy peach, Chunmi, fruit   | 11                 | 25 April 2017   | 18                          |
|              | Wuhan, Hubei              | Juicy peach, Zaoxianhong, fruit | 17            | 18 April 2017   | 20                          |
|              | Wuhan, Hubei              | Flat peach, Zaoyoupan, fruit | 12                 | 18 April 2017   | 20                          |
Table 6. Cont.

| Species                  | Location          | Host                        | Number of Isolates | Date       | Daily Mean Temperature (°C) |
|--------------------------|-------------------|-----------------------------|--------------------|------------|-----------------------------|
| Wuhan, Hubei             | Juicy peach, leaf | 9                           | 14 June 2017       | 25         |
| Xiaogan, Hubei           | Juicy peach, Chunmei, fruit | 4                   | 10 May 2017       | 20         |
| Qingzhen, Guizhou        | Juicy peach, Yingqing, fruit | 8                   | 21 August 2017    | 24         |
| Tongren, Guizhou         | Juicy peach, fruit | 2                           | 08 August 2018     | 29         |
| Guilin, Guangxi          | Juicy peach, Chunmi, fruit | 38                  | 18 May 2018       | 25         |
| Guilin, Guangxi          | Juicy peach, Chunmi, leaf | 4                   | 18 May 2018       | 25         |
| Fuzhou, Fujian           | Yellow peach, huangjinmi, fruit | 12                 | 27 July 2018      | 31         |
| Chengdu, Sichuan         | Zhongtaojinmi, fruit | 7                           | 28 June 2018      | 26         |
| C. siamense              | Qingdao, Shandong | Juicy peach, Yangjiaomi, fruit | 27               | 22 August 2017 | 27               |
| Shijiazhuang, Hebei      | Juicy peach, Dajibao, fruit | 14                 | 3 August 2018     | 30         |
| **Total**                |                   |                             |                    | 286        |

*The average of the daily mean temperatures on the sampling day and the previous six days.*

Figure 2. Prevalence of *Colletotrichum* spp. associated with peaches in China. (a) Map of the distribution of *Colletotrichum* spp. on peaches in China. Each color represents one *Colletotrichum* species, and the size of the circle indicates the number of isolates collected from that location. (b) Overall isolation rate (%) of *Colletotrichum* species; (c) number of sampling locations for each *Colletotrichum* species.
3.1. Phylogenetic Analyses

Phylogenetic trees were constructed based on the concatenated gene/locus sequences. MP and ML trees are not shown because the topologies were similar to the displayed BI tree (Figures 3–6). The number of taxa, aligned length (with gaps), invariable characters, uninformative variable characters, and phylogenetically informative characters of each gene/locus and combined sequences are listed in Tables 2–5.

For the *C. acutatum* species complex, in the multilocus sequence analyses (gene/locus boundaries in the alignment: ITS: 1–546, *GAPDH*: 551–815, *CHS-1*: 820–1101, *HIS3*: 1106–1492, *ACT*: 1497–1744, *TUB2*: 1749–2240) of 27 isolates from peaches in this study, 44 reference strains of *C. acutatum* species complex and one *Colletotrichum* species (*C. orchidophilum* strains CBS 632.80) as the outgroup, 2240 characters including the alignment gaps were processed. For the Bayesian analysis, a HKY + I model was selected for ITS, a HKY + G model for *GAPDH*, a K80 + I model for *CHS-1*, a GTR + I + G model for *HIS3*, and a GTR + G model for *ACT* and *TUB2*, and all were incorporated in the analysis (Table 2). As the phylogenetic tree shows in Figure 3, the 27 isolates of the *C. acutatum* species complex were clustered in three groups: 11 with *C. nymphaeae*, eight with *C. fioriniae*, and eight with *C. godetiae*. Although in the same general cluster, *C. nymphaeae* from China were genetically distinct from *C. nymphaeae* isolates from the USA and Brazil.

Figure 3. A Bayesian inference phylogenetic tree of 71 isolates in the *C. acutatum* species complex. *C. orchidophilum* (CBS 632.80) was used as the outgroup. The tree was built using combined sequences of the ITS, *GAPDH*, *CHS-1*, *HIS3*, *ACT*, and *TUB2*. BI posterior probability values (BI ≥ 0.70), MP bootstrap support values (MP ≥ 50%), and RAxML bootstrap support values (ML ≥ 50%) were shown at the nodes (BI/MP/ML). Tree length = 827, CI = 0.71, RI = 0.93, RC = 0.65, HI = 0.30. Ex-type isolates are in bold. Circles indicate isolates from fruits, and triangles indicate isolates from leaves.
Ex-type isolates are in bold. Circles indicate isolates from fruits, and triangles indicate isolates from leaves.

For the *C. gloeosporioides* species complex, DNA sequences of six genes/loci were obtained from 19 isolates from peaches in this study, with 42 reference isolates from the *C. gloeosporioides* species complex and the outgroup *C. boninense* CBS 123755. The gene/locus boundaries of the aligned 3034 characters (with gaps) were: ACT: 1–314, CAL: 319–1062, CHS-1: 1067–1366, GAPDH: 1371–1677, ITS: 1682–2295, TUB2: 2300–3034. For the Bayesian analysis, a HKY + G model was selected for ACT, a GTR + G model for CAL, a K80 + G model for CHS-1, a HKY + I model for GAPDH and TUB2, and a SYM + I + G model for ITS, and they were all incorporated in the analysis (Table 3). In the phylogenetic tree of the *C. gloeosporioides* species complex, 10 isolates clustered with *C. fructicola* and nine isolates clustered with *C. siamense* (Figure 4). They clustered together with isolates from South Korea and the USA.

**Figure 4.** A Bayesian inference phylogenetic tree of 61 isolates in the *C. gloeosporioides* species complex. *C. boninense* (CBS 123755) was used as the outgroup. The tree was built using combined sequences of the ACT, CAL, CHS-1, GAPDH, ITS, and TUB2. BI posterior probability values (BI ≥ 0.70), MP bootstrap support values (MP ≥ 50%), and RAxML bootstrap support values (ML ≥ 50%) were shown at the nodes (BI/MP/ML). Tree length = 1303, CI = 0.76, RI = 0.84, RC = 0.63, HI = 0.24. Ex-type strains are in bold. Circles indicate isolates from fruits, and triangles indicate isolates from leaves.
Figure 5. A Bayesian inference phylogenetic tree of 24 isolates in the *C. boninense* species complex. *C. gloeosporioides* (CBS 112999) was used as the outgroup. The tree was built using combined sequences of the ITS, GAPDH, *CHS*-1, *HIS3*, *ACT*, *TUB2* and *CAL*. BI posterior probability values (BI ≥ 0.70), MP bootstrap support values (MP ≥ 50%), and RAxML bootstrap support values (ML ≥ 50%) were shown at the nodes (BI/MP/ML). Tree length = 1404, CI = 0.76, RI = 0.79, RC = 0.60, HI = 0.24. Ex-type strains are in bold. Circles indicate isolates from fruits, and triangles indicate isolates from leaves.

For the *C. acutatum* species complex, in the multilocus sequence analyses (gene/locus boundaries in the alignment: ITS: 1–546, GAPDH: 551–815, *CHS*-1: 820–1101, *HIS3*: 1106–1492, *ACT*: 1497–1744, *TUB2*: 1749–2240) of 27 isolates from peaches in this study, 44 reference strains of *C. acutatum* species complex and one *Colletotrichum* species (*C. orchidophilum* strains CBS 632.80) as the outgroup, 2240 characters including the alignment gaps were processed. For the Bayesian analysis, a HKY + I model was selected for ITS, a HKY + G model for GAPDH, a K80 + I model for *CHS*-1, a GTR + I + G model for *HIS3*, and a GTR + G model for *ACT* and *TUB2*, and all were incorporated in the analysis (Table 2). As the phylogenetic tree shows in Figure 3, the 27 isolates of the *C. acutatum* species complex were clustered in three groups: 11 with *C. nymphaeae*, eight with *C. fioriniae*, and eight with *C. godetiae*. Although in the same general cluster, *C. nymphaeae* from China were genetically distinct from *C. nymphaeae* isolates from the USA and Brazil.
For the *C. gloeosporioides* species complex, DNA sequences of six genes/loci were obtained from 19 isolates from peaches in this study, with 42 reference isolates from the *C. gloeosporioides* species complex and the outgroup *C. boninense* CBS 123755. The gene/locus boundaries of the aligned 3034 characters (with gaps) were: *ACT* : 1–314, *CAL* : 319–1062, *CHS-1* : 1067–1366, *GAPDH* : 1371–1677, *ITS* : 1682–2295, *TUB2* : 2300–3034. For the Bayesian analysis, a HKY + G model was selected for *ACT*, a GTR + G model for *CAL*, a K80 + G model for *CHS-1*, a HKY + I model for *GAPDH* and *TUB2*, and a SYM + I + G model for *ITS*, and they were all incorporated in the analysis (Table 3). In the phylogenetic tree of the *C. gloeosporioides* species complex, 10 isolates clustered with...
C. fructicola and nine isolates clustered with C. siamense (Figure 4). They clustered together with isolates from South Korea and the USA.

Regarding the C. boninense species complex, in the multilocus analyses (gene/locus boundaries of ITS: 1–553, GAPDH: 558–843, CHS-1: 848–1127, HIS3: 1132–1524, ACT: 1529–1804, TUB2: 1809–2310, CAL: 2315–2763) of three isolates from peaches in this study, from 21 reference isolates of C. boninense species complex and one outgroup strain C. gloeosporioides CBS 112999, 2763 characters including the alignment gaps were processed. For the Bayesian analysis, a SYM + I + G model was selected for ITS, HKY + I for GAPDH and TUB2, K80 + G for CHS-1, GTR + I + G for HIS3, GTR + G for ACT, and HKY + G for CAL, and they were all incorporated in the analysis (Table 4). In Figure 5, three Chinese isolates clustered with C. karstii in the C. boninense species complex.

For the remaining phylogenetic analyses, the alignment of combined DNA sequences was obtained from 50 taxa, including two isolates from peaches in this study, 47 reference isolates of Colletotrichum species, and one outgroup strain Monilochaetes infuscans CBS 869.96. The gene/locus boundaries of the aligned 1981 characters (with gaps) were: ITS: 1–571, GAPDH: 576–896, CHS-1: 901–1165, ACT: 1170–1448, TUB2: 1453–1981. For the Bayesian analysis, a GTR + I + G model was selected for ITS and CHS-1, and HKY + I + G for GAPDH, ACT, and TUB2, and they were incorporated in the analysis (Table 5). In the phylogenetic tree, two isolates (YNHH2-2 and YNHH10-1 (CCTCC M 2020345)) clustered distantly from all known Colletotrichum species and are described herein as a new species, C. folicola (Figure 6). The PHI test result (Φw = 1) of C. folicola and its related species C. citrus-medicae ruled out the possibility of gene recombination interfering with the species delimitation (Figure 7). This is further evidence that C. folicola is a new species.

3.2. Taxonomy

Colletotrichum nymphaeae H.A. van der Aa, Netherlands Journal of Plant Pathology. 84: 110. (1978) (Figure 8).

---

**Figure 7.** PHI test of C. folicola and phylogenetically related species using both LogDet transformation and splits decomposition. PHI test value (Φw) < 0.05 indicate significant recombination within the datasets.

**3.2. Taxonomy**

Colletotrichum nymphaeae H.A. van der Aa, Netherlands Journal of Plant Pathology. 84: 110. (1978) (Figure 8).
Figure 7. PHI test of C. folicola and phylogenetically related species using both LogDet transformation and splits decomposition. PHI test value ($\Phi_w$) < 0.05 indicate significant recombination within the datasets.

3.2. Taxonomy

Colletotrichum nymphaeae H.A. van der Aa, Netherlands Journal of Plant Pathology. 84: 110. (1978) (Figure 8).

Description and illustration—Damm et al. [31].

Materials examined: China, Hubei province, Yichang city, on fruit of P. persica cv. NJC83, April 2017, Q. Tan, living culture HBYC1; Sichuan province, Chengdu city, on fruit of P. persica cv. Zhongtaojinmi, June 2018, Q. Tan, living culture SCCD 1; Fujian province, Fuzhou city, on fruit of P. persica cv. Huangjinmi, July 2018, Q. Tan, living culture FJFZ 1; Guangxi province, Guilin city, on leaves of P. persica cv. Chunmei, May 2018, Q. Tan, living culture GXGL 13-1; Guizhou province, Tongren city, on fruit of P. persica, June 2018, Q. Tan living culture GZTR 8-1; Hubei province, Jingmen city, on fruit of P. persica cv. NJC83, April 2018, Q. Tan, living culture HBJM 1-1; Hubei province, Wuhan city, on fruit of P. persica var. nucipersica cv. Zhongtaojinmi, April 2017, Q. Tan, living culture HBWH 2-1; ibid, on leaves of P. persica, June 2017, L.F. Yin, living culture HBWH 3-2; Hubei province, Xiaogan city, on fruit of P. persica cv. Chunmei, May 2017, Q. Tan, living culture HBXG 1.

Notes: Colletotrichum nymphaeae was first described on leaves of Nymphaea alba in Kortenhoef by Van der Aa [43]. C. nymphaeae is well separated from other species with TUB2, but all other genes have very high intraspecific variability [31]. Consistently, C. nymphaeae isolates collected in this study are different from ex-type strain CBS 515.78 in ITS (2 bp), GAPDH (1 bp), CHS-1 (3 bp), ACT (1 bp), HIS3 (3 bp), but with 100% identity in TUB2.

Colletotrichum fioriniae (Marcelino and Gouli) R.G. Shivas and Y.P. Tan, Fungal Diversity 39: 117. (2009) (Figure 9).
was also recovered from fruits of *C. godetiae* (*P. persica* (six-day-old PDA culture; (2018, Q. Tan, living culture GXGL 13-1; Guizhou province, Tongren city, on fruit of *P. persica*; (2018, Q. Tan, living culture FJFZ 1; Guangxi province, Guilin city, on leaves of *P. persica*), late JXJA 6; (2018, Q. Tan, living culture HBXG 1, HBWH 3-2; Hubei province, Xiaogan city, on fruit of *P. persica*), June 2018, Q. Tan living culture GZTR 7-1. Scale bars: (c) = 200 μm; (d–f) = 20 μm.

**Figure 9.** Biological characteristics of *Colletotrichum fioriniae*. (a,b) Front and back view of six-day-old PDA culture; (c) conidiomata; (d) conidia; (e) appressoria; (f) conidiophores ((a–e) isolate JXJA 6; (f) isolate JXJA 1). Scale bars: (c) = 200 μm; (d–f) = 20 μm.

Description and illustration—Damm et al. [31].

Materials examined: China, Jiangxi province, Jian city, on fruit of *P. persica*, August 2018, Q. Tan, living cultures JXJA 1, JXJA 6; Zhejiang province, Lishui city, on fruit of *P. persica*, September 2017, Q. Tan, living cultures ZJLS 1, ZJLS 11-1; Guizhou province, Tongren city, on fruit of *P. persica*, August 2018, Q. Tan, living culture GZTR 7-1.

Notes: *Colletotrichum acutatum* var. *fioriniae* was first isolated from *Fiorinia externa* [44] and host plants of the scale insect as an endophyte [45] in New York, USA. In 2009, Shivas and Tan identified it from *Acacia acuminate*, *Persea americana*, and *Mangifera indica* in Australia as a separate species and named it *Colletotrichum fioriniae* [46]. *C. fioriniae* was mainly isolated from wide host plants and fruits in the temperate zones [3,31]. In this study, the *C. fioriniae* isolates clustered in two subclades, which is consistent with the results of Damm’s study [31].

*Colletotrichum godetiae* P. Neergaard, *Friesia* 4: 72. (1950) (Figure 10).

Description and illustration—Damm et al. [31].

Materials examined: China, Yunnan Province, Honghe City, on leaves of *P. persica* cv. Hongxue, August 2017, Q. Tan, living cultures YNHH 1-1, YNHH 4-1, YNHH 6-1, YNHH 8-2 and YNHH 9-1.

Notes: *Colletotrichum godetiae* was first reported on the seeds of *Godetia hybrid* in Denmark by Neergaard in 1943 [47], and given detailed identification seven years later [48]. *C. godetiae* was also recovered from fruits of *Fragaria × ananassa*, *Prunus cerasus*, *Solanum betaceum*, *Citrus aurantium*, and *Olea europea* [49]; leaves of *Laurus nobilis* and *Mahonia aquifolium*; twigs of *Ugni molinae*; and canes of *Rubus idaeus* [31]. In this study, the isolates were obtained from peach leaves and could infect both the peach fruit and leaf.
in Australia as a separate species and named it *Colletotrichum fioriniae* [46]. *C. fioriniae* was mainly isolated from wide host plants and fruits in the temperate zones [3,31]. In this study, the *C. fioriniae* isolates clustered in two subclades, which is consistent with the results of Damm’s study [31].

*Colletotrichum godetiae* P. Neergaard, Friesia 4: 72. (1950) (Figure 10).

Description and illustration—Damm et al. [31].

**Figure 10.** Biological characteristics of *Colletotrichum godetiae*. (a,b) Front and back view of six-day-old PDA culture; (c) conidiomata; (d) conidia; (e–h) appressoria; (i) conidiophores ((a–f,i) isolate YNHH 1-1, (g,h) YNHH 9-1). Scale bars: (c) = 200 µm; (d–i) = 20 µm.

*Colletotrichum fructicola* H. Prihastuti et al., *Fungal Diversity* 39: 96. (2009) (Figure 11).

Description and illustration—Prihastuti et al. [50].

Materials examined: China, Guangdong province, Heyuan city, on fruit of *P. persica*, June 2017, Q. Tan, living culture GDHY 10-1; Guangdong province, Shaoguan city, on fruit of *P. persica* cv. Yingzuitao, August 2018, Q. Tan, living cultures GDSG 1-1, GDSG 5-1; Guizhou province, Tongren city, on fruit of *P. persica*, August 2018, Q. Tan, living culture GZTR 10-1.
honia aquifolium; twigs of Ugni molinae; and canes of Rubus idaeus [31]. In this study, the isolates were obtained from peach leaves and could infect both the peach fruit and leaf. Colletotrichum fructicola H. Prihastuti et al., Fungal Diversity 39: 96. (2009) (Figure 11).

Description and illustration—Prihastuti et al. [50].

Figure 11. Biological characteristics of Colletotrichum fructicola. (a,b) Front and back view of six-day-old PDA culture; (c) conidiomata; (d) conidia; (e) appressoria; (f) conidiophores; (g) ascomata; (h,i) asci; (j) ascospores ((a–e) isolate GDHY 10-1; (f–j) isolate GDSG 1-1). Scale bars: (c) = 200 µm; (d–j) = 20 µm.

Notes: Colletotrichum fructicola was first described from the berries of Coffea arabica in Chiang Mai Province, Thailand [50]. Subsequently, C. fructicola was reported on a wide range of hosts including Malus domestica, Fragaria × ananassa, Limonium sinuatum, Pyrus pyrifolia, Dioscorea alata, Theobroma cacao Vaccinium spp., Vitis vinifera, and Prunus persica [3,51]. In this study, the conidia and ascospores of C. fructicola isolates (9.3–18.9 × 3.4–8.2 µm, mean ± SD = 14.3 ± 1.7 × 5.6 ± 0.5 µm; 12.6–22.0 × 3.1–7.6 µm, mean ± SD = 17.3 ± 0.5 × 5.0 ± 0.5 µm) (Table S3) were larger than that of ex-type (MFLU 090228, ICMP 185819: 9.7–14 × 3–4.3 µm, mean ± SD = 11.53 ± 1.03 × 3.55 ± 0.32 µm; 9–14 × 3–4 µm, mean ± SD = 11.91 ± 1.38 × 3.32 ± 0.35 µm).
Colletotrichum siamense H. Prihastuti et al., *Fungal Diversity* 39: 98. (2009) (Figure 12).

Figure 12. Biological characteristics of *Colletotrichum siamense*. (a,b) Front and back view of six-day-old PDA culture; (c) conidiomata; (d) conidia; (e) appressoria; (f) conidiophores ((a–e) isolate SDQD10-1; (f) isolate HBSJZ 1-1). Scale bars: (c) = 200 μm; (d–f) = 20 μm.

Description and illustration—Prihastuti et al. [50].

Materials examined: China, Shandong province, Qingdao city, on fruit of *P. persica* cv. Yangjiaomi, August 2017, Q. Tan, living cultures SDQD 1-1, SDQD 10-1; Hebei province, Shijiazhuang city, on fruit of *P. persica* cv. Dajiubao, August 2018, Q. Tan, living cultures HBSJZ 1-1, HBSJZ 3-1.

Notes: *Colletotrichum siamense* was first identified on the berries of *Coffea arabica* in Chiang Mai Province, Thailand [50] and reported to have a wide range of hosts across several tropical, subtropical, and temperate regions, including *Persea americana* and *Carica papaya* in South Africa; *Fragaria × ananassa*, *Vitis vinifera*, and *Malus domestica* in the USA; *Hymenocallis americana* and *Pyrus pyrifolia* in China; etc. [3,8,51]. In this study, we collected *C. siamense* isolates from the temperate zone in China; the conidia (13.2–18.3 × 4.6–6.3 μm, mean ± SD = 15.3 ± 0.4 × 5.4 ± 0.3 μm) (Table S3) were larger than those of the ex-holotype (MFLU 090228, ICMP 185819: 7–18.3 × 3–4.3 μm, mean ± SD = 10.18 ± 1.74 × 3.46 ± 0.36 μm).

*Colletotrichum karsti* Y.L. Yang et al., *Cryptogamie Mycologie*. 32: 241. (2011) (Figure 13).

Description and illustration—Yang et al. [52].

Materials examined: China, Yunnan province, Honghe city, on leaves of *P. persica* cv. Hongxue, August 2017, Q. Tan, living cultures YNHH 3-1, YNHH 3-2, and YNHH 5-2.

Notes: *Colletotrichum karsti* was first described from *Vanda* sp. (*Orchidaceae*) as a pathogen on diseased leaf and endophyte of roots in Guizhou province, China [52]. *C. karsti* is the most common and geographically diverse species in the *C. boninense* species complex, and occurs on wild hosts including *Vitis vinifera*, *Capsicum* spp., *Lycopersicon esculentum*, *Coffea* sp., *Citrus* spp., *Musa banksia*, *Passiflora edulis*, *Solanum betaceum*, *Zamia obliqua*, *P. persica* cv. Dajiubao, August 2018, Q. Tan, living cultures HBSJZ 1-1, HBSJZ 3-1.
In this study, the conidia of *C. karsti* isolates (10.6–14.9 × 5.8–7.4 μm, mean ± SD = 12.9 ± 0.3 × 6.7 ± 0.2 μm) (Table S3) were smaller than those of the ex-holotype (CGMCC3.14194: 12–19.5 × 5–7.5 μm, mean ± SD = 15.4 ± 1.3 × 6.5 ± 0.5 μm).

*Colletotrichum folicola* Q. Tan and C.X. Luo, sp. nov. (Figure 14).

**Figure 13.** Biological characteristics of *Colletotrichum karsti*. (a,b) Front and back view of six-day-old PDA culture; (c) conidiomata; (d) conidia; (e) appressoria; (f) conidiophores; (g) ascomata; (h,i) asci; (j) ascospores ((a–j) isolate YNHH 3-1). Scale bars: (c) = 200 μm; (d–j) = 20 μm.

*Colletotrichum folicola* Q. Tan and C.X. Luo, sp. nov. (Figure 14).
Type: China, Yunnan Province, Honghe City, on leaves of *Prunus persica* cv. Hongxue, August 2017, Q. Tan. Holotype YNHH 10-1, Ex-type culture CCTCC M 2020345.

Sexual morphs were not observed. Asexual morphs developed on PDA. Vegetative hyphae were hyaline, smooth-walled, septate, and branched. Chlamydospores were not observed. Conidiomata acervular, conidiophores, and setae formed on hyphae or brown to black stromata. Conidiomata color ranged from yellow to grayish-yellow to light brown. Setae were medium brown to dark brown, smooth-walled, 2–6 septa, 50–140 μm long, base cylindrical, 2.5–4.5 μm in diameter at the widest part, with tip acute. Conidiophores were

MycoBank Number: MB843363.

Etymology: Referring to the host organ from which the fungus was collected.

Type: China, Yunnan Province, Honghe City, on leaves of *Prunus persica* cv. Hongxue, August 2017, Q. Tan. Holotype YNHH 10-1, Ex-type culture CCTCC M 2020345.

Sexual morphs were not observed. Asexual morphs developed on PDA. Vegetative hyphae were hyaline, smooth-walled, septate, and branched. Chlamydospores were not observed. Conidiomata acervular, conidiophores, and setae formed on hyphae or brown to black stromata. Conidiomata color ranged from yellow to grayish-yellow to light brown. Setae were medium brown to dark brown, smooth-walled, 2–6 septa, 50–140 μm long, base cylindrical, 2.5–4.5 μm in diameter at the widest part, with tip acute. Conidiophores were

---

**Figure 14.** Biological characteristics of *Colletotrichum folicola*. (a,b) Front and back view of six-day-old PDA culture; (c,d) conidiomata; (e) setae; (f) conidia; (g) appressoria; (h) conidiophores ((a–h) isolate YNHH 10-1). Scale bars: (c,d) = 200 μm; (e–g) = 20 μm; (h,i) = 10 μm.
hyaline to pale brown, smooth-walled, septate, and up to 55 µm long. Conidiogenous cells were hyaline, cylindrical, 12.3–14.5 × 4.4–6.3 µm, with an opening of 1.8–2.5 µm. Conidia were straight, hyaline, aseptate, cylindrical, and had a round end, 12.3–15.4 × 5.6–7.8 µm, mean ± SD = 13.6 ± 0.1 × 6.5 ± 0.3 µm, L/W ratio = 2.1. Appressoria were single, dark brown, elliptical to clavate, 5.6–13.7 × 4.0–8.2 µm, mean ± SD = 8.4 ± 0.5 × 5.9 ± 0.1 µm, L/W ratio = 1.4.

Culture characteristics: Colonies on PDA attained 16–21 mm diameter in three days at 25 °C and 7–10 mm diameter in three days at 30 °C; greenish-black, white at the margin, and aerial mycelium scarce.

Additional specimens examined: China, Yunnan Province, Honghe City, on leaves of Prunus persica cv. Hongxue, August 2017, Q. Tan, living culture YNHH 2-2.

Notes: Colletotrichum folicola is phylogenetically most closely related to C. citrus-medicae (Figure 6). The PHI test (Φw = 1) revealed no significant recombination between C. folicola and C. citrus-medicae (Figure 7), which was described from diseased leaves of Citrus medica in Kunming, Yunnan Province, China [54]. C. folicola is different from C. citrus-medicae holotype isolate HGUP 1554 in ITS (with 99.04% sequence identity), GAPDH (99.13%), CHS-1 (98.44%), and HIS3 (99.72%). The sequence data of ACT do not separate the two species. In terms of morphology, C. folicola differs from C. citrus-medicae by having setae, smaller conidia (12.3–15.4 × 5.6–7.8 µm vs. 13.5–17 × 5.5–9 µm), longer appressoria (5.6–13.7 × 4.0–8.2 µm vs. 6–9.5 × 5.5–8.5 µm), and colonies that are greenish-black rather than white and pale brownish as in C. citrus-medicae.

3.3. Pathogenicity Tests

Pathogenicity tests were conducted to confirm Koch’s postulates on fruit and leaves for all species identified (Table S4; Figures 15 and 16). Colletotrichum species collected in this study showed high diversity in virulence. C. nymphaeae, C. fioriniae, C. fructicola, and C. siamense, which were already reported to be pathogens of peaches, were pathogenic on both peach leaves and fruit. C. fructicola and C. siamense from the C. gloeosporioides species complex were more virulent compared to species from the C. acutatum species complex. Interestingly, C. folicola and C. karsti showed tissue-specific pathogenicity. Isolates of these two species were all collected from leaves, and mainly infected leaves in the pathogenicity test. C. folicola did not infect peach fruit at all, and the size of lesions on leaves was comparably small (0.20 ± 0.06 cm). C. karsti did infect peach fruit, but the infection rate was only around 20% (7/36 isolates) and the size of lesions was 0.06 ± 0.01 cm. In contrast, the infection rate on leaves was 63.9% (23/36 isolates) and the lesion size was 0.35 ± 0.13 cm. Isolates of C. godetiae collected from peach leaves in Yunnan province were virulent on both leaves and fruit, with the leaf and fruit infection rates and lesion diameters being 88.3% (53/60 isolates) and 0.54 ± 0.05 cm and 90% (54/60 isolates) and 0.50 ± 0.17 cm, respectively (Table S4; Figure 16).
Figure 15. Symptoms of peach fruits and leaves induced by inoculation of spore suspensions of seven *Colletotrichum* spp. after six days at 25 °C. (a) Symptoms resulting from H$_2$O, isolates HBYC 1, JXJA 6, and YNHH 1-1 (left to right). (b) Symptoms resulting from isolates GDHY 10-1, SDQD 10-1, YNHH3-1, and YNHH10-1 (left to right).
4. Discussion

This study is the first large-scale investigation of Colletotrichum species causing anthracnose fruit and leaf diseases in peaches in China. The most common Colletotrichum species were C. nymphaeae and C. fioriniae of the C. acutatum species complex and C. fruticola and C. siamense of the C. gloeosporioides species complex. The same species were also identified in the southeastern USA [17,21,22], where a shift over time appeared to favor C. gloeosporioides species complex in South Carolina. The authors speculated that inherent resistance of C. acutatum to benzimidazole fungicides (MBCs) may have given this species complex a competitive advantage when MBCs were frequently used [22]. As MBCs were replaced by other fungicides (including quinone outside inhibitors and demethylation inhibitors), that competitive advantage may have disappeared and C. gloeosporioides species may have increased in prevalence [22,55]. In support of this hypothesis is previous research showing a higher virulence of C. gloeosporioides on peaches, pears, and apples compared to C. acutatum [8,56,57].

Also, this study and others show that the C. gloeosporioides species complex may be better adapted to the hot South Carolina climate compared to the C. acutatum species complex [3]. MBCs are still popular fungicides in Chinese peach production regions. Therefore, it is possible that the dominance of C. acutatum species complex, specifically C. nymphaeae is, at least in part, a result of fungicide selection.

The high prevalence of C. nymphaeae in Chinese peach orchards is consistent with other local studies reporting the same species affecting a wide variety of other fruit crops in China. For example, C. nymphaeae was reported in Sichuan province on blueberries and loquats [58,59], in Hubei province on strawberries and grapevines [60,61], and in Zhejiang
province on pecans [62]. Internationally, it is one of the most common species affecting pome fruits, stone fruits, and small fruits [23,63,64].

*C. godetiae*, *C. karsti*, and *C. folicola* were reported on peaches for the first time. The three species were geographically isolated and only present in Yunnan province. Rare occurrences of *Colletotrichum* species have also been formerly observed on peaches, i.e., *C. truncatum* was only found in one of many orchards examined in South Carolina, USA [25]. *C. godetiae* and *C. karsti* are well-known pathogens of fruit crops. *C. godetiae* was reported to cause disease on apples, strawberries, and grapes [65–68], while *C. karsti* was reported to affect apples and blueberries [69,70]. It is, therefore, possible that these pathogens migrated from other hosts into Yunnan province peach orchards. The observed occurrence, however, does point to either a rather rare host transfer event or to environmental conditions that favor these species. Yunnan province is located in southwestern China and peach production is popular in the Yunnan–Guizhou high plateau, a region with low latitude and high altitude [71]. The complicated local topography and diverse climate lead to highly abundant biodiversity [72], which may explain the emergence of the new species *C. folicola*.

As mentioned above, regional differences in *Colletotrichum* species composition in commercial orchards may be influenced by fungicide selection pressure. For example, *C. acutatum* is less sensitive to benomyl, thiophanate-methyl, and other MBC fungicides compared with *C. gloeosporioides* [56,73,74]. Meanwhile, all *C. nymphaeae* strains in this study have been confirmed to be resistant to carbendazim (MBC) [75]. *C. nymphaeae* was reported to be less sensitive to demethylation inhibitor (DMIs) fungicides (flutriafol and fenbuconazole) compared with *C. fioriniae*, *C. fructicola*, and *C. siamense* [21] and *C. gloeosporioides* was reported to be inherently tolerant to fludioxonil [76,77]. Most of the peach farms in China are small and there is vast diversity in the approaches to managing diseases. However, MBC (i.e., carbendazim and thiophanate-methyl) fungicides are commonly used to control peach diseases, followed by DMIs (i.e., difenoconazole). Whether fungicide selection had an impact on the *Colletotrichum* species distribution is unknown, but the high prevalence of *C. acutatum* species complex and their resilience to MBCs (and, in the case of *C. nymphaeae*, to DMIs) would allow for such a hypothesis.

In conclusion, this study provides the morphological, molecular, and pathological characterization of seven *Colletotrichum* spp. occurring on peaches in China. This is of great significance for the prevention and control of anthracnose disease in different areas in China.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jof8030313/s1, Table S1: Primers used in this study, with sequences, annealing temperature, and sources [51,78–85]; Table S2: Isolates of seven *Colletotrichum* species collected from peaches in China, with details about host tissue, location, and GenBank accession number; Table S3: The sizes of conidia, appresoria, ascospores, and mycelial growth rate of the representative isolates of *Colletotrichum* spp. obtained in this study; Table S4: Infection rates of seven *Colletotrichum* spp. inoculated on peach fruit and leaves.

**Author Contributions:** Conceptualization, Q.T., G.S. and C.-X.L.; methodology, Q.T., C.C., W.-X.Y., L.-F.Y. and C.-X.L.; software, Q.T. and C.C.; validation, Q.T. and C.-X.L.; formal analysis, Q.T. and G.S.; investigation, Q.T., L.-F.Y. and C.-X.L.; data curation, Q.T. and C.-X.L.; writing—original draft preparation, Q.T., G.S. and C.-X.L.; writing—review and editing, G.S., C.C. and C.-X.L.; visualization, Q.T. and C.C.; supervision, W.-X.Y. and C.-X.L.; project administration and funding acquisition, C.-X.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the China Agriculture Research System of the Ministry of Finance and the Ministry of Agriculture and Rural Affairs (CARS-30-3-03), and the Fundamental Research Funds for the Central Universities (No. 2662020ZKY018).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.
Data Availability Statement: Alignments generated during the current study are available from TreeBASE (http://treebase.org/treebase-web/home.html; study 29227). All sequence data are available in the NCBI GenBank, following the accession numbers in the manuscript.

Acknowledgments: We sincerely thank the reviewers for their contributions during the revision process.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Zheng, Y.; Crawford, G.W.; Chen, X. Archaeological evidence for peach (Prunus persica) cultivation and domestication in China. *PLoS ONE* **2014**, *9*, e106595. [CrossRef] [PubMed]
2. Food and Agricultural Organization of the United Nations (FAOSTAT) Website. 2020. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 14 February 2022).
3. Dowling, M.; Peres, N.; Villani, S.; Schnabel, G. Managing *Colletotrichum* on fruit crops: A "complex" challenge. *Plant Dis.* **2020**, *104*, 2301–2316. [CrossRef] [PubMed]
4. Dowling, M.; Schnabel, G. Understanding plant diseases using art and technology. *Int. J. Fruit Sci.* **2016**, *20*, 959–966. [CrossRef]
5. Stensvand, A.; Borve, J.; Talgo, V. Overwintering diseased plant parts and newly infected flowers and fruit as sources of inoculum for *Colletotrichum acutatum* in sour cherry. *Plant Dis.* **2017**, *101*, 1207–1213. [CrossRef] [PubMed]
6. Sutton, T.B.; Shane, W.W. Epidemiology of the perfect stage of *Gloverella cinigulata* on apples. *Phytopathology* **1983**, *73*, 1179–1183. [CrossRef]
7. De Silva, D.D.; Crous, P.W.; Ades, P.K.; Hyde, K.D.; Taylor, P.W.J. Life styles of *Colletotrichum* species and implications for plant biosecurity. *Fungal Biol. Rev.* **2017**, *31*, 155–168. [CrossRef]
8. Fu, M.; Crous, P.W.; Bai, Q.; Zhang, P.F.; Xiang, J.; Guo, Y.S.; Zhao, F.F.; Yang, M.M.; Hong, N.; Xu, W.X.; et al. *Colletotrichum* species associated with anthracnose of *Pyrus* spp. in China. *Persoonia* **2019**, *42*, 1–35. [CrossRef]
9. Sutton, B.C. The Coelomycetes. *Fungi Imperfecti with Pycnidia, Acervuli and Stromata*; Commonwealth Mycological Institute: Kew, UK, 1980.
10. Cacciola, S.O.; Gilardi, G.;Faedda, R.; Schena, L.; Pane, A.; Garibaldi, A.; Gullino, M.L. Characterization of *Colletotrichum ocimi* population associated with black spot of sweet basil (*Ocimum basilicum*) in Northern Italy. *Plants* **2020**, *9*, 654. [CrossRef]
11. Riolo, M.; Aloi, F.; Pane, A.; Cara, M.; Cacciola, S.O. Twig and shoot dieback of *Citrus*, a new disease caused by *Colletotrichum* species. *Cells* **2021**, *10*, 449. [CrossRef]
12. Cai, L.; Hyde, K.D.; Taylor, P.W.J.; Weir, B.S.; Waller, J.M.; Abang, M.M.; Zhang, J.Z.; Yang, Y.L.; Phoulivong, S.; Liu, Z.Y.; et al. A polyphasic approach for studying *Colletotrichum*. *Fungal Divers* **2009**, *39*, 183–204.
13. Liu, F.; Wang, M.; Damm, U.; Crous, P.W.; Cai, L. Species boundaries in plant pathogenic fungi: A *Colletotrichum* case study. *BMC Evol. Biol.* **2016**, *16*, 14. [CrossRef] [PubMed]
14. Talhinhas, P.; Baroncelli, R. *Colletotrichum* species and complexes: Geographic distribution, host range and conservation status. *Fungal Divers.* **2021**, *110*, 109–198. [CrossRef]
15. Yu, Z.; Jiang, X.; Zheng, H.; Zhang, H.; Qiao, M. Fourteen new species of foliar *Colletotrichum* associated with the invasive plant *Ageratina adenophora* and surrounding crops. *J. Fungi* **2022**, *8*, 185. [CrossRef] [PubMed]
16. Zheng, H.; Yu, Z.; Jiang, X.; Fang, L.; Qiao, M. Endophytic *Colletotrichum* species from aquatic plants in southwest China. *J. Fungi* **2022**, *8*, 87. [CrossRef] [PubMed]
17. Bernstein, B.; Zehb, E.I.; Dean, R.A.; Shabi, E. Characteristics of *Colletotrichum* from peach, apple, pecan, and other hosts. *Plant Dis.* **1995**, *79*, 478–482. [CrossRef]
18. Adaskaveg, J.E.; Hartin, R.J. Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. *Phytopathology* **1997**, *87*, 979–987. [CrossRef]
19. Schnabel, G.; Chai, W.; Cox, K.D. Identifying and characterizing summer diseases on ‘Babygold’ peach in South Carolina. *Plant Health. Prog.* **2006**, *7*, 30. [CrossRef]
20. Kim, W.G.; Hong, S.K. Occurrence of anthracnose on peach tree caused by *Colletotrichum* species. *Plant Pathol. J.* **2008**, *24*, 80–83. [CrossRef]
21. Chen, S.N.; Luo, C.X.; Hu, M.J.; Schnabel, G. Sensitivity of *Colletotrichum* species, including *C. fioriniae* and *C. nymphaeae*, from peach to demethylating inhibitor fungicides. *Plant Dis.* **2016**, *100*, 2434–2441. [CrossRef]
22. Hu, M.J.; Grabke, A.; Schnabel, G. Investigation of the *Colletotrichum gloeosporioides* species complex causing peach anthracnose in South Carolina. *Plant Dis.* **2015**, *99*, 797–805. [CrossRef]
23. Moreira, R.R.; Silva, G.A.; De Mio, L.L.M. *Colletotrichum acutatum* complex causing anthracnose on peach in Brazil. *Austral. Plant Pathol.* **2020**, *49*, 179–189. [CrossRef]
24. Lee, D.M.; Hassan, O.; Chang, T. Identification, characterization, and pathogenicity of *Colletotrichum* species causing anthracnose of peach in Korea. *Mycobiology* **2020**, *48*, 210–218. [CrossRef]
55. Hu, M.J.; Grabke, A.; Dowling, M.E.; Holstein, H.J.; Schnabel, G. Resistance in *Colletotrichum siamense* from peach and blueberry to thiophanate-methyl and azoxystrobin. *Plant Dis.* 2015, 99, 806–814. [CrossRef]

56. Munir, M.; Amsden, B.; Dixon, E.; Vaillancourt, L.; Gauthier, N.A.W. Characterization of *Colletotrichum* species causing bitter rot of apple in Kentucky orchards. *Plant Dis.* 2016, 100, 2194–2203. [CrossRef]

57. Eaton, M.J.; Edwards, S.; Inocencio, H.A.; Machado, F.J.; Nuckles, E.M.; Farman, M.; Gauthier, N.A.; Vaillancourt, L.J. Diversity and cross-infection potential of *Colletotrichum* causing fruit rots in mixed-fruit orchards in Kentucky. *Plant Dis.* 2021, 105, 1115–1128. [CrossRef] [PubMed]

58. Zhang, Y.B.; Meng, K.; Shu, J.P.; Zhang, W.; Wang, H.J. First report of anthracnose on pecan (*Carya illinoensis*) caused by *Colletotrichum nymphaeae* on loquat fruit in China. *Plant Dis.* 2018, 102, 243. [CrossRef]

59. Liu, X.; Zheng, X.; Khaskheli, M.I.; Sun, X.; Chang, X.; Gong, G. Identification of *Colletotrichum* species associated with blueberry anthracnose in Sichuan, China. *Pathogens* 2020, 9, 718. [CrossRef] [PubMed]

60. Han, Y.C.; Zeng, X.G.; Xiang, F.Y.; Ren, L.; Chen, F.Y.; Gu, Y.C. Distribution and characteristics of *Colletotrichum* species associated with anthracnose of raspberry in Hebei, China. *Plant Dis.* 2016, 100, 996–1006. [CrossRef]

61. Liu, M.; Zhang, W.; Zou, Y.; Liu, Y.; Yan, J.Y.; Li, X.H.; Jayawardena, R.S.; Hyde, K.D. First report of twig anthracnose on grapevine caused by *Colletotrichum nymphaeae* in China. *Plant Dis.* 2016, 100, 2530. [CrossRef]

62. Zhang, Y.B.; Meng, K.; Shu, J.P.; Zhang, W.; Wang, H.J. First report of anthracnose on pecan (*Carya illinoensis*) caused by *Colletotrichum nymphaeae* in China. *Plant Dis.* 2019, 103, 1432–1433. [CrossRef]

63. Braganca, C.A.D.; Damm, U.; Baroncelli, R.; Massola, N.S.; Crous, P.W. Species of the *Colletotrichum acutatum* complex associated with anthracnose diseases of fruit in Brazil. *Fungal Biol.* 2016, 120, 547–561. [CrossRef]

64. Wang, N.Y.; Forcelini, B.B.; Peres, N.A. Anthracnose fruit and root necrosis of strawberry are caused by a dominant species within the *Colletotrichum acutatum* species complex in the United States. *Phytopathology* 2019, 109, 1293–1301. [CrossRef]

65. Baroncelli, R.; Sreenivasaprasad, S.; Thor, M.R.; Sukno, S.A. First report of apple bitter rot caused by *Colletotrichum godetiae* in the United Kingdom. *Plant Dis.* 2014, 98, 1000–1001. [CrossRef]

66. Munda, A. First report of *Colletotrichum fariniae* and *C. godetiae* causing apple bitter rot in Slovenia. *Plant Dis.* 2014, 98, 1282. [CrossRef]

67. Zapparata, A.; Da Lio, D.; Sarrocco, S.; Vannacci, G.; Baroncelli, R. First report of *Colletotrichum godetiae* causing grape (*Vitis vinifera*) berry rot in Italy. *Plant Dis.* 2017, 101, 1051–1052. [CrossRef]

68. Karimi, K.; Arzaniou, M.; Pertot, I. Weeds as potential inoculum reservoir for *Colletotrichum nymphaeae* causing strawberry anthracnose in Iran and rec-PCR fingerprinting as useful marker to differentiate *C. acutatum* complex on strawberry. *Front. Microbiol.* 2019, 10, 13. [CrossRef] [PubMed]

69. Rios, J.A.; Pinho, D.B.; Moreira, W.R.; Pereira, O.L.; Rodrigues, F.A. First report of *Colletotrichum karstii* causing anthracnose on blueberry leaves in Brazil. *Plant Dis.* 2015, 99, 157–158. [CrossRef] [PubMed]

70. Velho, A.C.; Stadnik, M.J.; Wallhead, M. Unraveling *Colletotrichum* species associated with Glomerella leaf spot of apple. *Trop. Plant Pathol.* 2019, 44, 197–204. [CrossRef]

71. Yang, W.; Zhang, X. A discussion on structural adjustment of fruit cultivation in Yunnan. *Southeast China J. Agric. Sci.* 2003, 16, 103–106.

72. Yang, Y.M.; Tian, K.; Hao, J.M.; Pei, S.J.; Yang, Y.X. Biodiversity and biodiversity conservation in Yunnan, China. *Biodivers. Conserv.* 2004, 13, 813–826. [CrossRef]

73. Peres, N.A.R.; Souza, N.L.; Peever, T.L.; Timmer, L.W. Benomyl sensitivity of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* from citrus. *Plant Dis.* 2004, 88, 125–130. [CrossRef]

74. Chung, W.H.; Ishii, H.; Nishimura, K.; Fukaya, M.; Yano, K.; Kajitani, Y. Fungicide sensitivity and phylogenetic relationship of anthracnose fungi isolated from various fruit crops in Japan. *Plant Dis.* 2006, 90, 506–512. [CrossRef]

75. Usman, H.M.; Tan, Q.; Fan, F.; Karim, M.M.; Yin, W.X.; Zhu, F.X.; Luo, C.X. Sensitivity of *Colletotrichum nymphaeae* to six fungicides and characterization of fludioxonil resistant isolates in China. *Plant Dis.* 2021, 106, 165–173. [CrossRef]

76. Schnabel, G.; Tan, Q.; Schneider, V.; Ishii, H. Inherent tolerance of *Colletotrichum gloeosporioides* to fludioxonil. *Pest. Biochem. Physiol.* 2021, 172, 6. [CrossRef] [PubMed]

77. Usman, H.M.; Tan, Q.; Karim, M.M.; Adnan, M.; Yin, W.X.; Zhu, F.X.; Luo, C.X. Sensitivity of *C. fructicola* and *C. siamense* of peach in China to multiple classes of fungicides and characterization of pyraclostrobin-resistant isolates. *Plant Dis.* 2021, 105, 3459–3465. [CrossRef] [PubMed]

78. Gardes, M.; Bruns, T.D. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 1993, 2, 113–118. [CrossRef] [PubMed]

79. White, T.; Bruns, T.; Lee, S.; Taylor, J.; Innis, M.; Gelfand, D.; Sninsky, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Academic Press: Cambridge, MA, USA, 1990; pp. 315–322.

80. Guerber, J.C.; Liu, B.; Correll, J.C.; Johnston, P.R. Characterization of diversity in *Colletotrichum acutatum sensu lato* by sequence analysis of two gene introns, mtDNA and intron RFLPs, and mating compatibility. *Mycolologia* 2003, 95, 872–895. [CrossRef] [PubMed]

81. Carbone, I.; Kohn, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycolologia* 1999, 91, 553–556. [CrossRef]
82. Woudenberg, J.H.C.; Aveskamp, M.M.; de Gruyter, J.; Spiers, A.G.; Crous, P.W. Multiple Didymella teleomorphs are linked to the Phoma clematidina morphotype. Persoonia 2009, 22, 56–62. [CrossRef]

83. O’Donnell, K.; Cigelnik, E. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusarium are nonorthologous. Mol. Phylogenet. Evol. 1997, 7, 103–116. [CrossRef]

84. Glass, N.L.; Donaldson, G.C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. 1995, 61, 1323–1330. [CrossRef]

85. Crous, P.W.; Groenewald, J.Z.; Risede, J.M.; Simoneau, P.; Hywel-Jones, N.L. Calonectria species and their Cylindrocladium anamorphs: Species with sphaeropedunculate vesicles. Stud. Mycol. 2004, 50, 415–430.