



High species diversity in *Diaporthe* associated with citrus diseases in China

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Key words

Citrus diseases
Diaporthe
new taxa
pathogenicity
phylogenetic analysis

Abstract Species in *Diaporthe* have broad host ranges and cosmopolitan geographic distributions, occurring as endophytes, saprobes and plant pathogens. Previous studies have indicated that many *Diaporthe* species are associated with *Citrus*. To further determine the diversity of *Diaporthe* species associated with citrus diseases in China, we conducted extensive surveys in major citrus-producing areas from 2017–2020. Diseased tissues were collected from leaves, fruits, twigs, branches and trunks showing a range of symptoms including melanose, dieback, gummosis, wood decay and canker. Based on phylogenetic comparisons of DNA sequences of the internal transcribed spacer regions (ITS), calmodulin (*cal*), histone H3 (*his3*), translation elongation factor 1-alpha (*tef1*) and beta-tubulin (*tub2*), 393 isolates from 10 provinces were identified as belonging to 36 species of *Diaporthe*, including 32 known species, namely *D. apiculata*, *D. biconispora*, *D. biguttulata*, *D. caryae*, *D. citri*, *D. citriasiana*, *D. compacta*, *D. discoidispora*, *D. endophytica*, *D. eres*, *D. fusicola*, *D. fulvicolor*, *D. guangxiensis*, *D. hongkongensis*, *D. hubeiensis*, *D. limonicola*, *D. litchii*, *D. novem*, *D. passifloricola*, *D. penetriteum*, *D. pescicola*, *D. pometiiae*, *D. sackstonii*, *D. sennicola*, *D. sojiae*, *D. spinosa*, *D. subclavata*, *D. tectonae*, *D. tibetensis*, *D. unshiuensis*, *D. velutina* and *D. xishuangbanica*, and four new species, namely *D. gammata*, *D. jishouensis*, *D. ruiiliensis* and *D. sexualispora*. Among the 32 known species, 14 are reported for the first time on *Citrus*, and two are newly reported from China. Among the 36 species, *D. citri* was the dominant species as exemplified by its high frequency of isolation and virulence. Pathogenicity tests indicated that most *Diaporthe* species obtained in this study were weakly aggressive or non-pathogenic to the tested citrus varieties. Only *D. citri* produced the longest lesion lengths on citrus shoots and induced melanose on citrus leaves. These results further demonstrated that a rich diversity of *Diaporthe* species occupy *Citrus*, but only a few species are harmful and *D. citri* is the main pathogen for *Citrus* in China. The present study provides a basis from which targeted monitoring, prevention and control measures can be developed.

Citation: Xiao XE, Liu YD, Zheng F, et al. 2023. High species diversity in *Diaporthe* associated with citrus diseases in China.

Persoonia 51: 229–256. <https://doi.org/10.3767/persoonia.2023.51.06>.

Effectively published online: 27 November 2023 [Received: 17 February 2023; Accepted: 8 September 2023].

INTRODUCTION

Citrus is one of the most important fruit crops in the world. China represents one of the centres of origin for *Citrus*, with more than 4 000 years of cultivation history (Deng et al. 2008, Wu et al. 2018, Guo et al. 2019). *Citrus* is widely grown in southern China with a production of 46.7 M tons in 2021 (FAO 2021), making China the largest producer of *Citrus*. However, fungal diseases associated with *Citrus*, especially dieback, gummosis, canker, melanose and stem-end rot caused by *Diaporthe* species, are commonly encountered in orchards. These diseases can severely impact *Citrus* production, including affecting tree

vigour or even resulting in tree death, as well as reducing fruit production and marketability (Cai et al. 2011, Chen et al. 2014).

The genus *Diaporthe* (syn. *Phomopsis*) was established by Nitschke (1870) with *D. eres* as the type species. Species in this genus have a cosmopolitan distribution and broad host range, occurring as endophytes, saprobes and plant pathogens (Santos & Phillips 2009, Santos et al. 2011, Udayanga et al. 2011, 2012, 2015, Gomes et al. 2013, Dissanayake et al. 2017, Yang et al. 2018). The identification of *Diaporthe* has traditionally relied mainly on host associations and morphological characteristics such as shape and size of ascospores, conidiomata, conidia and conidiophores (Wehmeyer 1933, Uecker 1988, Rehner & Uecker 1994, Mostert et al. 2001a, Udayanga et al. 2011). Previous studies have identified more than 2 000 species names as belonging to *Diaporthe* and *Phomopsis* (Index Fungorum, <http://www.indexfungorum.org/>, accessed 25 July 2022). However, there has been some controversy over the reliance on host tree association and morphological features for species identifications in this genus. Indeed, studies have shown that morphological features for many *Diaporthe* species are not always stable, as they may change in response to environmental changes (Wehmeyer 1933, Nitimargi 1935, Gomes et al. 2013). With the development of molecular phylogenetics, Rehner & Uecker (1994) used inter-

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nal transcribed spacer sequences (ITS1 and ITS2) to conduct a phylogenetic analysis of *Diaporthe* isolates from different hosts and regions. These results further demonstrated that species in *Diaporthe* were not host-specific, but that many species have broad host ranges that overlap with each other. Since then, molecular identification has been widely applied in *Diaporthe* (Uddin et al. 1998, Mostert et al. 2001a, b, Murali et al. 2006, Van Rensburg et al. 2006, Santos et al. 2011, Udayanga et al. 2012, Gomes et al. 2013). A polyphasic approach based on a combination of morphological characteristics, multi-locus phylogenetic and phytopathological analyses is commonly used for the identification of *Diaporthe* species (Guarnaccia & Crous 2017, 2018, Guarnaccia et al. 2018, Guo et al. 2020). In phylogenetic analyses, five loci, namely the internal transcribed spacer regions (ITS), calmodulin (*cal*), histone H3 (*his3*), translation elongation factor 1-alpha (*tef1*) and beta-tubulin (*tub2*), have been considered as the optimal combination for *Diaporthe* species delimitation (Santos et al. 2017), and have been commonly used for molecular identification in recent studies (Guarnaccia & Crous 2017, 2018, Guarnaccia et al. 2018, Yang et al. 2018, Manawasinghe et al. 2019, Guo et al. 2020, Sun et al. 2021).

Previous studies have shown that many species of *Diaporthe* are associated with *Citrus*. *Diaporthe citri* represents one of the most important pathogens in *Citrus*, causing gummosis or blight of perennial branches and trunks, stem-end rot of fruits, and melanose of fruits, young leaves and shoots (Mondal et al. 2007, Huang et al. 2013, Udayanga et al. 2014b). Of these symptoms, the most concerning is melanose, which affects all *Citrus* species and occurs in most citrus-growing regions worldwide (Timmer et al. 2000, Huang et al. 2013, Udayanga et al. 2014a, Guarnaccia & Crous 2018, Chaisiri et al. 2022). Based on morphology, multigene phylogeny (ITS, *cal*, *tef1* and *tub2*) and pathogenicity traits of *Diaporthe* isolates obtained from citrus fruits and shoots, Huang et al. (2013) revealed that *D. citri* was the dominant species responsible for citrus melanose in China, and two new taxa (*D. citriasiana* and *D. citri-chinensis*) were described. However, isolates in that study mainly came from citrus melanose tissues, and seldom from diseased branches. In a subsequent study, Huang et al. (2015) obtained eight known and seven novel *Diaporthe* species from asymptomatic citrus tissues in China, suggesting a high species diversity of *Diaporthe* in China. Based on DNA sequences from ITS, *cal*, *his3*, *tef1* and *tub2* gene regions, Guarnaccia & Crous (2017) conducted a large-scale survey for citrus branch diseases in Europe and reported that three known and two newly described *Diaporthe* species were associated with *Citrus* in Europe. Pathogenicity tests demonstrated that these five species exhibited different levels of aggressiveness. While *D. citri* was not reported in this survey, it was found associated with shoot blight on *Citrus reticulata* in another survey conducted on small islands of Portugal (Guarnaccia & Crous 2018). Based on DNA sequences at five gene loci (ITS, *cal*, *his3*, *tef1* and *tub2*), Chaisiri (2018) reported 22 known *Diaporthe* species obtained from citrus tissues with melanose symptoms in Jiangxi Province, China, 13 of which represented first reports on citrus. However, pathogenicity results revealed only *D. passifloricola* as pathogenic to citrus fruits, while the pathogenicity status of the other species remained unresolved. In a recent study, Cui et al. (2021) reported two *Diaporthe* species (*D. taoicola* and *D. siamensis*) identified by morphology and multi-locus phylogeny of ITS, *tef1* and *tub2*, on *C. sinensis* causing fruit rot in China, and their pathogenicity was confirmed through artificial inoculation on healthy fruits of *C. sinensis*. Similarly, Dong et al. (2021) identified 11 endophytic *Diaporthe* species, including two novel species, isolated from healthy tissues of *C. grandis* (Table 1).

Based on these findings, it is clear that a rich and diverse assembly of *Diaporthe* species occur on *Citrus* in China. Indeed, the fact that Chaisiri (2018) isolated so many *Diaporthe* species from melanose samples in Jiangxi province alone suggests that there are likely more undiscovered *Diaporthe* species on *Citrus*. In addition, most attention was paid to the symptoms of melanose on young leaves, shoots and fruits (Huang et al. 2013, Chaisiri et al. 2021, Zeng et al. 2021), and few studies have addressed the species diversity of *Diaporthe* on infected citrus branches in China. Moreover, previous surveys have been limited in scope, or had relatively small sample sizes. Considering the expansion of citrus planting areas and the replacement of citrus varieties in China in recent years, *Diaporthe* species and populations on *Citrus* may have also changed over time. Furthermore, the pathogenicity of most reported species has not been tested, and their potential economic impact on *Citrus* remains unknown. During 2017–2020, a large-scale survey of citrus diseases was therefore conducted in all major citrus production regions in China. The host investigated included all species in *Citrus* genus in China, and allied genera (*Fortunella*, *Poncirus*) in the *Rutaceae* family. The objectives of this study were to: a) identify the species of *Diaporthe* associated with citrus diseases, especially branch diseases based on morphological traits and phylogenetic analysis; b) identify the dominant species associated with *Citrus* diseases; and c) determine the potential risk to *Citrus* of *Diaporthe* species other than *D. citri*.

MATERIALS AND METHODS

Sampling and isolation

From 2017 to 2020, diseased citrus samples showing dieback, decay, canker and gummosis of twigs, branches and trunks, and melanose of fruits, leaves and shoots were collected from the major citrus-cultivation regions in 10 provinces of China, including Chongqing, Fujian, Guangdong, Guizhou, Hunan, Jiangxi, Shanghai, Sichuan, Yunnan and Zhejiang. The investigated citrus species included *C. limon*, *C. maxima*, *C. reticulata*, *C. sinensis*, *Fortunella margarita*, *Poncirus trifoliata* and hybrids. Whenever possible, multiple samples of diseased tissues at each site and each host tree species were collected. The isolation of fungal strains was conducted following the protocol of Xiao et al. (2021). Type specimens of new species were deposited in Zhejiang University, and ex-type living cultures were deposited in the China General Microbiological Culture Collection Centre (CGMCC), Beijing, China.

DNA extraction, PCR amplification and sequencing

Isolates used for DNA extraction were grown on potato dextrose agar (PDA, 200 g potatoes, 20 g glucose and 15 g agar/L water) and incubated at 25 °C. Genomic DNA was extracted from axenic cultures according to the Cetyl trimethylammonium bromide (CTAB) method (Van Burik et al. 1998). Partial regions of five loci were amplified. The internal transcribed spacers (ITS) of the nuclear ribosomal gene cluster were amplified with primers ITS1 and ITS4 (White et al. 1990). Part of the calmodulin (*cal*) was amplified with primers CAL228F and CAL737R (Carbone & Kohn 1999); histone H3 gene (*his3*) was amplified with primers CYLH3F (Crous et al. 2004) and H3-1b (Glass & Donaldson 1995); the translation elongation factor 1-alpha gene (*tef1*) was amplified with primers EF1-688F (Alves et al. 2008) or EF1-728F and EF1-986R (Carbone & Kohn 1999), and the beta-tubulin gene (*tub2*) was amplified with Bt2a and Bt2b (Glass & Donaldson 1995). All amplification reactions were performed in a total volume of 25 µL mixture consisted of 12.5 µL of 2× Taq Master Mix (Dye Plus) (Vazyme), 9.5 µL ddH₂O, 1 µL of each forward and reverse primer, and 1 µL DNA template.

Table 1 *Diaporthe* species associated with *Citrus*.

| Species | Host | Organ | Symptom | Location | Pathogenicity to <i>Citrus</i> | References |
|-------------------------------------|---|---------------------|--|-----------------|--------------------------------|---|
| <i>D. apiculata</i> | <i>C. sinensis</i> , <i>C. reticulata</i> | Leaf | Melanose | China | Not performed ^d | Chaisiri (2018) |
| | <i>C. grandis</i> | Twig, fruit | Non-symptom | China | Not performed | Dong et al. (2021) |
| | <i>C. sinensis</i> , <i>C. reticulata</i> | Leaf | Melanose | China | Pathogenic | This study |
| <i>D. aquatica</i> | <i>C. grandis</i> | Fruit | Non-symptom | China | Not performed | Dong et al. (2021) |
| <i>D. arecae</i> | <i>Citrus</i> sp. | Fruit | Unknown | India, Suriname | Not performed | Gomes et al. (2013) |
| | <i>C. grandis</i> | Twig | Non-symptom | China | Not performed | Dong et al. (2021) |
| | <i>Citrus</i> spp. | Twig, branch, leaf | Non-symptom, citrus scab | China | Not performed | Huang et al. (2015) |
| | <i>Citrus</i> spp. | Leaf, twig, fruit | Dieback, melanose | China | Pathogenic | This study |
| <i>D. baccae</i> | <i>Citrus</i> spp. | Twig, branch, trunk | Dieback, canker | Italy | Pathogenic | Guarnaccia & Crous (2017) |
| <i>D. biconispora</i> | <i>Fortunella margarita</i> , <i>C. sinensis</i> , <i>C. grandis</i> | Branch | Non-symptom | China | Not performed | Huang et al. (2015) |
| | <i>C. grandis</i> | Leaf | Non-symptom | China | Not performed | Dong et al. (2021) |
| | <i>C. sinensis</i> | Fruit | Melanose | China | Pathogenic | This study |
| <i>D. biguttulata</i> | <i>C. limon</i> | Branch | Non-symptom | China | Not performed | Huang et al. (2015) |
| | <i>C. reticulata</i> , <i>C. sinensis</i> | Leaf | Melanose | China | Pathogenic | This study |
| <i>D. caryae</i> ^b | <i>C. reticulata</i> | Twig | Dieback | China | Pathogenic | This study |
| <i>D. citri</i> | <i>Citrus</i> spp. | Leaf, branch, fruit | Melanose, resin disease, stem-end rot | Worldwide | Pathogenic | Timmer et al. (2000), Huang et al. (2013), Udayanga et al. (2014b), Guarnaccia & Crous (2018), this study |
| <i>D. citriasiana</i> | <i>C. grandis</i> , <i>C. unshiu</i> | Leaf, branch | Anonymous spot, dead wood | China | Pathogenic | Huang et al. (2013) |
| <i>D. citrichinensis</i> | <i>C. unshiu</i> , <i>C. grandis</i> , <i>Fortunella margarita</i> | Branch, leaf | Dead wood, citrus scab, non-symptom | China | Non-pathogenic | Huang et al. (2013) |
| <i>D. compacta</i> ^b | <i>C. reticulata</i> , <i>C. sinensis</i> , <i>C. unshiu</i> | Leaf, branch | Dieback, melanose | China | Pathogenic | This study |
| <i>D. cytospora</i> ^a | <i>C. limon</i> , <i>C. sinensis</i> | Twig | Unknown | Spain, USA | Not performed | Udayanga et al. (2014b) |
| <i>D. discoidispora</i> | <i>C. sinensis</i> , <i>C. unshiu</i> | Twig | Non-symptom | China | Not performed | Huang et al. (2015) |
| | <i>Citrus</i> spp. | Leaf, fruit | Melanose | China | Pathogenic | This study |
| <i>D. endocitricola</i> | <i>C. grandis</i> | Fruit | Non-symptom | China | Not performed | Dong et al. (2021) |
| <i>D. endophytica</i> | <i>C. unshiu</i> | Twig | Citrus scab | China | Not performed | Huang et al. (2015) |
| <i>D. eres</i> | <i>Citrus</i> spp. | Twig, branch, fruit | Non-symptom | China | Not performed | Huang et al. (2015) |
| | <i>Citrus</i> spp. | Twig, branch, fruit | Dieback, gummosis, melanose, non-symptom | China | Pathogenic | This study |
| <i>D. eucalyptorum</i> | <i>C. sinensis</i> | Leaf | Melanose | China | Not performed | Chaisiri (2018) |
| <i>D. foeniculina</i> | <i>C. limon</i> | Fruit | Unknown | Spain, USA | Not performed | Udayanga et al. (2014) |
| | <i>Citrus</i> spp. | Twig, branch, trunk | Dieback, canker | Europe | Pathogenic | Guarnaccia & Crous (2017) |
| | <i>Citrus</i> spp. | Twig, branch | Dieback, canker | Greece | Pathogenic | Vakalounakis et al. (2019) |
| | <i>C. limon</i> | Fruit | Fruit rot | Turkey | Pathogenic | Tekiner et al. (2020) |
| <i>D. fulvicolor</i> ^b | <i>C. limon</i> , citrus hybrid | Twig | Dieback | China | Pathogenic | This study |
| <i>D. fuscicola</i> ^b | <i>C. sinensis</i> | Leaf | Melanose | China | Pathogenic | This study |
| <i>D. gammata</i> ^a | <i>C. reticulata</i> | Twig, leaf | Dieback, melanose | China | Pathogenic | This study |
| <i>D. guangdongensis</i> | <i>C. grandis</i> | Fruit | Non-symptom | China | Not performed | Dong et al. (2021) |
| <i>D. guangxiensis</i> ^b | <i>C. sinensis</i> | Fruit | Melanose | China | Pathogenic | This study |
| <i>D. hongkongensis</i> | <i>Citrus</i> spp. | Leaf, twig | Non-symptom | China | Not performed | Huang et al. (2015) |
| | <i>Citrus</i> spp. | Leaf, twig, fruit | Dieback, melanose | China | Pathogenic | This study |
| <i>D. hubeiensis</i> ^b | <i>C. reticulata</i> , <i>C. sinensis</i> | Leaf | Melanose | China | Pathogenic | This study |
| <i>D. inferilis</i> | <i>C. sinensis</i> | Fruit | Decaying | Suriname | Not performed | Guarnaccia & Crous (2017) |
| <i>D. jishouensis</i> ^a | <i>Citrus</i> spp. | Leaf, twig, fruit | Dieback, melanose | China | Pathogenic | This study |
| <i>D. lagerstromiae</i> | <i>C. sinensis</i> | Leaf | Melanose | China | Not performed | Chaisiri (2018) |

Table 1 (cont.)

| Species | Host | Organ | Symptom | Location | Pathogenicity to Citrus | References |
|---------------------------------------|--|---------------------|-------------------|----------|-------------------------|---------------------------|
| <i>D. limonicola</i> | <i>C. limon</i> | Branch | Canker | Malta | Pathogenic | Guarnaccia & Crous (2017) |
| | <i>C. grandis</i> | Leaf | Non-symptom | China | Not performed | Dong et al. (2021) |
| | <i>C. unshiu</i> | Branch | Gummosis | China | Pathogenic | This study |
| | <i>Litchi chinensis</i> | Leaf | Diseased | China | Not performed | Sun et al. (2021) |
| <i>D. litchii</i> ^b | <i>C. reticulata</i> | Leaf | Melanose | China | Not performed | Chaisiri (2018) |
| <i>D. mali</i> | <i>C. grandis</i> | Twig | Non-symptom | China | Not performed | Dong et al. (2021) |
| <i>D. masirevicii</i> | <i>C. limon</i> | Branch | Canker | Malta | Pathogenic | Guarnaccia & Crous (2017) |
| <i>D. melitensis</i> | <i>C. grandis</i> | Branch | Non-symptom | China | Not performed | Huang et al. (2015) |
| <i>D. multifurcata</i> | <i>C. aurantiifolia</i> , <i>C. japonica</i> | Twig | Dieback | Italy | Pathogenic | Guarnaccia & Crous (2017) |
| <i>D. novem</i> ^c | Citrus hybrid | Twig | Dieback | China | Pathogenic | This study |
| <i>D. ovalispora</i> | <i>C. limon</i> | Twig | Non-symptom | China | Not performed | Huang et al. (2015) |
| <i>D. passifloricola</i> | <i>C. reticulata</i> | Fruit | Melanose | China | Not performed | Chaisiri (2018) |
| | <i>C. grandis</i> | Fruit, twig | Non-symptom | China | Not performed | Dong et al. (2021) |
| | <i>C. maxima</i> | Leaf | Melanose | China | Pathogenic | This study |
| <i>D. penetratrum</i> | <i>C. sinensis</i> | Fruit | Melanose | China | Not performed | Chaisiri (2018) |
| | <i>C. reticulata</i> | Fruit | Melanose | China | Pathogenic | This study |
| <i>D. perniciosa</i> | <i>C. reticulata</i> | Leaf | Melanose | China | Not performed | Chaisiri (2018) |
| <i>D. perseae</i> | <i>C. grandis</i> | Leaf | Non-symptom | China | Not performed | Dong et al. (2021) |
| <i>D. pescicola</i> ^b | <i>C. reticulata</i> | Fruit | Melanose | China | Pathogenic | This study |
| <i>D. podocarpi-macrophylli</i> | <i>C. sinensis</i> | Twig, leaf | Melanose | China | Pathogenic | Chaisiri (2018) |
| <i>D. pometae</i> ^b | <i>Pometia pinnata</i> | Leaf | Diseased | China | Not performed | Huang et al. (2021) |
| | <i>C. maxima</i> | Leaf | Melanose | China | Pathogenic | This study |
| <i>D. ruii</i> ^a | <i>C. limon</i> | Twig | Dieback | China | Pathogenic | This study |
| <i>D. sackstonii</i> | <i>C. reticulata</i> | Fruit | Melanose | China | Not performed | Chaisiri (2018) |
| | <i>C. sinensis</i> , <i>C. unshiu</i> | Fruit | Melanose | China | Pathogenic | This study |
| <i>D. sennae</i> | <i>C. grandis</i> | Leaf | Non-symptom | China | Not performed | Dong et al. (2021) |
| <i>D. sennicola</i> | <i>C. sinensis</i> | Fruit | Melanose | China | Not performed | Chaisiri (2018) |
| | <i>C. maxima</i> | Twig | Melanose | China | Pathogenic | This study |
| <i>D. sexualispora</i> ^a | <i>C. unshiu</i> | Fruit | Melanose | China | Pathogenic | This study |
| <i>D. siamensis</i> | <i>C. sinensis</i> | Fruit | Unknown | China | Pathogenic | Cui et al. (2021) |
| <i>D. sojae</i> | <i>C. limon</i> , <i>C. reticulata</i> | Twig, branch, leaf | Non-symptom | China | Not performed | Huang et al. (2015) |
| | <i>Citrus</i> spp. | Leaf, twig, fruit | Dieback, melanose | China | Pathogenic | This study |
| <i>D. spinosa</i> ^b | <i>C. unshiu</i> | Fruit | Melanose | China | Pathogenic | This study |
| <i>D. subclavata</i> | <i>C. unshiu</i> | Leaf | Citrus scab | China | Not performed | Huang et al. (2015) |
| | <i>C. maxima</i> , <i>C. reticulata</i> , <i>C. sinensis</i> | Leaf, fruit | Melanose | China | Pathogenic | This study |
| <i>D. taicola</i> | <i>C. sinensis</i> | Fruit | Unknown | China | Pathogenic | Cui et al. (2021) |
| <i>D. tectoriae</i> ^{b,c} | <i>C. sinensis</i> | Fruit | Melanose | China | Pathogenic | This study |
| <i>D. tectonendophytica</i> | <i>C. sinensis</i> | Fruit | Melanose | China | Not performed | Chaisiri (2018) |
| <i>D. tibetensis</i> ^b | <i>C. sinensis</i> | Leaf | Melanose | China | Pathogenic | This study |
| <i>D. unshiuensis</i> | <i>C. sinensis</i> , <i>Fortunella margarita</i> | Fruit, branch, twig | Non-symptom | China | Not performed | Huang et al. (2015) |
| | <i>Citrus</i> spp. | Leaf, shoot, fruit | Dieback, melanose | China | Pathogenic | This study |
| <i>D. velutina</i> ^b | <i>C. reticulata</i> , <i>C. sinensis</i> , <i>C. unshiu</i> | Leaf, twig | Dieback, melanose | China | Pathogenic | This study |
| <i>D. xishuangbanica</i> ^b | <i>C. maxima</i> | Leaf | Melanose | China | Pathogenic | This study |

^a Novel species in this study.^b Species reported for the first time on *Citrus* in this study.^c Species reported for the first time from China.^d Not performed means no pathogenicity tests conducted on *Citrus*.

The amplification conditions consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at a suitable temperature for 30 s (55 °C for ITS, 54 °C for *cal*, 57 °C for *his3*, 55 °C for *tef1* and *tub2*), and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. The PCR products were sent to Shangya Biotechnology (Hangzhou, China) for Sanger DNA sequencing. The nucleotide sequences were assembled and edited with MEGA v. 7.0.26 (Kumar et al. 2016). Sequences obtained in this study were deposited in the GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov>; Table 2).

Phylogenetic analyses

Sequences obtained in this study were generated and compared against the NCBI GenBank nucleotide datasets (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to determine the closest relatives for a taxonomic framework of the studied isolates. Alignments of the five individual loci (ITS, *cal*, *his3*, *tef1*, *tub2*), including sequences obtained from this study and those downloaded from GenBank (Table 3), were initially performed by using MAFFT v. 7 online service (<https://mafft.cbrc.jp/alignment/server/index.html>) (Kato et al. 2019), with iterative refinement methods (FFT-NS-i), and then manually edited with MEGA v. 7.0.26 software.

Two separate phylogenetic analyses were conducted based on concatenated loci for the *D. eres* species complex and the remaining *Diaporthe* spp. included in this study. Because of heterogeneity among copies of the ITS sequences within strains of the *D. eres* species complex, the analysis of species within this species complex may lead to overestimation of species diversity (Udayanga et al. 2014a). Therefore, only the *cal*, *his3*, *tef1* and *tub2* sequences were combined for the phylogenetic analyses of the *D. eres* species complex. Phylogenetic analyses of the remaining species were performed for combined sequences of ITS, *cal*, *his3*, *tef1* and *tub2*. The Incongruence Length Difference test (ILD) was conducted in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002) to determine if a conflict existed among these four or five loci datasets were concatenated (Farris et al. 1994, Cunningham 1997). Bayesian inference (BI) analyses were conducted using MrBayes v. 3.2.7 (Ronquist & Huelsenbeck 2003), and MrModeltest v. 2.4 (Nylander 2004) was used to determine the best nucleotide substitution model for each partition. The Markov Chain Monte Carlo (MCMC) analyses used four simultaneous chains and started from random trees for 100 000 000 generations and trees were sampled every 10 generations. Analyses stopped automatically when standard deviation of split frequencies fell below 0.01. The burn-in fraction was set to 0.25, after which the 50 % majority rule consensus trees and posterior probability (PP) values were calculated.

Maximum-likelihood (ML) analyses for each dataset were performed on the multi-locus alignment using IQtree v. 2.1.3 (Nguyen et al. 2015), and selected the GTR site substitution model to conduct analyses. Bootstrap analysis was conducted with 1000 replicates to evaluate branch support (Hillis & Bull 1993). For both BI and ML analyses, the phylogenetic trees were viewed in FigTree v. 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree>). Aligned datasets and phylogenetic trees were deposited in TreeBASE (<http://treebase.org>; study number S30134).

Morphology

To observe sporocarps, we induced sporulation by inoculating isolates onto 2 % PDA, and 2 % tap water agar supplemented with sterile pine needles (PNA) (Smith et al. 1996) and alfalfa stems (ASA) (Udayanga et al. 2014b). Cultures were incubated

at 25 °C in 12/12 h fluorescent light/dark cycle. Sporocarps were embedded in a Leica Biosystem Tissue Freezing Medium (Leica Biosystems Nussloch GmbH, Nussloch, Germany) and sectioned (5–8 µm thick) using a freezing microtome (CryoStar NX50 HOP, Thermo Fisher Scientific, Walldorf, Germany) at -20 °C (Chen et al. 2018). Morphological observations of reproductive structures were examined with a compound microscope (Eclipse 80i, Nikon, Japan) and images were recorded with a Nikon digital camera (NIS-Elements F3.0, Nikon, Japan). Measurements were made with Fiji-ImageJ software (Schindelin et al. 2012). Fifty conidia were measured per isolate, and 30 measurements were taken of other morphological structures. Colony morphology was characterised from cultures grown on PDA, malt extract agar (MEA, 15 g malt extract and 15 g agar/L water) and oatmeal agar (OA, 30 g oatmeal and 15 g agar/L water) after 15 d at 25 °C in 12/12 h fluorescent light/dark cycle, and colony colours were determined according to the colour charts of Rayner (1970).

Pathogenicity tests

Two representative isolates from each identified *Diaporthe* species, except for those with only one isolate, were selected for pathogenicity testing in this study. Given that *Diaporthe* is widely distributed on citrus, citrus varieties were randomly selected for pathogenicity tests. Inoculation tests were conducted both in shoots and leaves. For shoot inoculation, the pathogenicity test was conducted in vitro first. Healthy green shoots (0.6–1.5 cm diam) were collected from *C. paradisi* trees and *C. reticulata* cv. ponkan trees, and 10 shoots were inoculated with each isolate. Shoots were superficially wounded between two nodes using a cork borer (5 mm diam). Inoculations were conducted by placing a 7-d-old, 5 mm diam mycelial plug from the margins of colonies on a wound. The inoculated area was covered with Parafilm. The negative control treatment was inoculated with sterile PDA plugs. The inoculated shoots were covered with paraffin at their ends to prevent desiccation and incubated at 25 °C in moist chambers. Following inoculation, the disease incidences were calculated and the internal lesions or wound lengths were measured. Data were analysed by one-way analysis of variance (ANOVA) using SPSS Statistics 20 software (SPSS 2011).

Based on the results of in vitro inoculation, isolates with different pathogenicity were selected for in vivo inoculation on healthy plants of *C. limon*, citrus hybrid cv. cocktail grapefruit and *F. margarita*. Each representative isolate, as well as the control, was inoculated onto five shoots. After 10 d, the symptoms were assessed.

Pathogenicity tests on leaves were performed on seedlings of *C. tangerina* cv. Hongjv, plants of *C. limon* and *C. sinensis*. Based on the pathogenicity test results of shoots, only moderate to highly aggressive isolates were selected for testing on leaves. Conidial suspensions were used for inoculation, at a concentration of 10⁵–10⁶ conidia/mL. Firstly, alcohol-soaked cottonwool was used to disinfect the leaf surface. After air drying, conidial suspensions were spread on the leaf surface, and finally the inoculated seedlings were each enclosed in a plastic bag to ensure high humidity, and the bag was removed after 3 d. The negative control treatment was sprayed with sterile water. Three seedlings or shoots with newly generated leaves were inoculated for each isolate. To confirm Koch's postulates, the inoculated fungi were re-isolated by cutting small pieces of necrotic tissue from the edges of each lesion and plating them onto PDA plates at 25 °C. Species identities were confirmed based on morphology.

Table 2 Details of *Diaporthe* isolates studied.

| Species ^a | Isolate | Location | Host | Associated symptoms | GenBank accession number ^b | | | | | |
|-----------------------|-------------------------|------------------------|--------------------------------|---------------------------|---------------------------------------|----------|----------|----------|----------|----------|
| | | | | | ITS | cal | his3 | tef1 | tub2 | |
| <i>D. apiculata</i> | ZJUE 0357 | Congjiang, Guizhou | <i>C. reticulata</i> | Leaf melanose | OP218106 | OP265433 | OP265497 | OP265561 | OP265625 | |
| | ZJUE 0358 | Congjiang, Guizhou | <i>C. reticulata</i> | Leaf melanose | OP218107 | OP265434 | OP265498 | OP265562 | OP265626 | |
| | ZJUE 0388 | Chenzhou, Hunan | <i>C. sinensis</i> cv. Newhall | Leaf melanose | OP218108 | OP265435 | OP265499 | OP265563 | OP265627 | |
| | ZJUE 0429 | Yichun, Jiangxi | <i>C. reticulata</i> | Leaf melanose | OP218109 | OP265436 | OP265500 | OP265564 | OP265628 | |
| | ZJUE 0425 | Ganzhou, Jiangxi | <i>C. sinensis</i> cv. Newhall | Fruit melanose | OP218117 | OP265444 | OP265508 | OP265572 | OP265636 | |
| <i>D. biconispora</i> | ZJUE 0369 | Luxi, Hunan | <i>C. sinensis</i> cv. Newhall | Leaf melanose | OP218118 | OP265445 | OP265509 | OP265573 | OP265637 | |
| | ZJUE 0398 | Luxi, Hunan | <i>C. reticulata</i> | Leaf melanose | OP218119 | OP265446 | OP265510 | OP265574 | OP265638 | |
| | ZJUE 0408 | Luxi, Hunan | <i>C. reticulata</i> | Leaf melanose | OP218120 | OP265447 | OP265511 | OP265575 | OP265639 | |
| | ZJUE 0276 | Jishou, Hunan | <i>C. reticulata</i> | Trunk dieback | ON035561 | ON221719 | ON113059 | ON049537 | ON221770 | |
| <i>D. citri</i> | ZJUE 0281 | Jishou, Hunan | <i>C. reticulata</i> | Twig dieback | ON035562 | ON221720 | ON113060 | ON049538 | ON221771 | |
| | ZJUE 0154 | Linhai, Zhejiang | <i>C. unshiu</i> | Branch dieback and crack | ON035564 | ON221722 | ON113062 | ON049540 | ON221773 | |
| | ZJUE 0223 | Ningbo, Zhejiang | <i>C. unshiu</i> | Branch dieback and crack | ON035565 | ON221723 | ON113063 | ON049541 | ON221774 | |
| | ZJUE 0254 | Changxingdao, Shanghai | Hybrid cv. Hongmeiren | Branch decay and gummosis | ON035566 | ON221724 | ON113064 | ON049542 | ON221775 | |
| | ZJUE 0274 | Jishou, Hunan | <i>C. reticulata</i> | Branch dieback and crack | ON035567 | ON221725 | ON113065 | ON049543 | ON221776 | |
| | ZJUE 0294 | Xingan, Jiangxi | <i>C. reticulata</i> | Twig dieback | ON035568 | ON221726 | ON113066 | ON049544 | ON221777 | |
| | ZJUE 0297 | Nanfeng, Jiangxi | <i>C. reticulata</i> | Twig dieback | ON035569 | ON221727 | ON113067 | ON049545 | ON221778 | |
| | ZJUE 0306 | Binchuan, Yunan | Hybrid cv. Wogan | Twig dieback | ON035570 | ON221728 | ON113068 | ON049546 | ON221779 | |
| | ZJUE 0383 | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218122 | OP265449 | OP265513 | OP265577 | OP265641 | |
| | ZJUE 0412 | Shaoyang, Hunan | <i>C. unshiu</i> | Fruit melanose | OP218123 | OP265450 | OP265514 | OP265578 | OP265642 | |
| | ZJUE 0413 | Shaoyang, Hunan | <i>C. unshiu</i> | Fruit melanose | OP218124 | OP265451 | OP265515 | OP265579 | OP265643 | |
| | ZJUE 0428 | Yichun, Jiangxi | <i>C. reticulata</i> | Leaf melanose | OP218125 | OP265452 | OP265516 | OP265580 | OP265644 | |
| | <i>D. citriasiana</i> | ZJUE 0217 | Ningbo, Zhejiang | Hybrid cv. Hongmeiren | Shoot blight | ON035571 | ON221729 | ON113069 | ON049547 | ON221780 |
| | | ZJUE 0286 | Changde, Hunan | <i>C. unshiu</i> | Twig dieback | ON035572 | ON221730 | ON113070 | ON049548 | ON221781 |
| | | ZJUE 0362 | Chenzhou, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218126 | OP265453 | OP265517 | OP265581 | OP265645 |
| | | ZJUE 0416 | Shaoyang, Hunan | <i>C. unshiu</i> | Leaf melanose | OP218127 | OP265454 | OP265518 | OP265582 | OP265646 |
| | | ZJUE 0431 | Quzhou, Zhejiang | <i>C. paradisi</i> | Leaf melanose | OP218128 | OP265455 | OP265519 | OP265583 | OP265647 |
| ZJUE 0269 | | Jishou, Hunan | <i>C. unshiu</i> | Twig dieback | ON035573 | ON221731 | ON113071 | ON049549 | ON221782 | |
| ZJUE 0270 | | Jishou, Hunan | <i>C. unshiu</i> | Twig dieback | ON035574 | ON221732 | ON113072 | ON049550 | ON221783 | |
| <i>D. compacta</i> | ZJUE 0271 | Jishou, Hunan | <i>C. unshiu</i> | Branch dieback | ON035575 | ON221733 | ON113073 | ON049551 | ON221784 | |
| | ZJUE 0272 | Jishou, Hunan | <i>C. unshiu</i> | Twig dieback | ON035576 | ON221734 | ON113074 | ON049552 | ON221785 | |
| | ZJUE 0380 | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218129 | OP265456 | OP265520 | OP265584 | OP265648 | |
| | ZJUE 0403 | Luxi, Hunan | <i>C. reticulata</i> | Leaf melanose | OP218130 | OP265457 | OP265521 | OP265585 | OP265649 | |
| | ZJUE 0384 | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218131 | OP265458 | OP265522 | OP265586 | OP265650 | |
| | ZJUE 0410 | Luxi, Hunan | <i>C. reticulata</i> | Leaf melanose | OP218132 | OP265459 | OP265523 | OP265587 | OP265651 | |
| | ZJUE 0417 | Shaoyang, Hunan | <i>C. unshiu</i> | Leaf melanose | OP218133 | OP265460 | OP265524 | OP265588 | OP265652 | |
| | <i>D. discoidispora</i> | ZJUE 0312 | Tailong, Chongqing | <i>C. limon</i> | Twig dieback | QQ703343 | QQ719547 | QQ719561 | QQ719575 | QQ719589 |
| | | ZJUE 0313 | Tailong, Chongqing | <i>C. limon</i> | Twig dieback | QQ703344 | QQ719548 | QQ719562 | QQ719576 | QQ719590 |
| | | ZJUE 0351 | Zhangzhou, Fujian | <i>C. maxima</i> | Leaf melanose | QQ703345 | QQ719549 | QQ719563 | QQ719577 | QQ719591 |
| ZJUE 0148 | | Linhai, Zhejiang | <i>C. unshiu</i> | Twig dieback | ON035577 | ON221735 | ON113075 | ON049553 | ON221786 | |
| ZJUE 0149 | | Linhai, Zhejiang | <i>C. unshiu</i> | Branch dieback | ON035578 | ON221736 | ON113076 | ON049554 | ON221787 | |
| <i>D. endophytica</i> | ZJUE 0156 | Linhai, Zhejiang | <i>C. unshiu</i> | Twig dieback | ON035579 | ON221737 | ON113077 | ON049555 | ON221788 | |
| | ZJUE 0162 | Quzhou, Zhejiang | <i>C. maxima</i> | Branch decay and gummosis | ON035580 | ON221738 | ON113078 | ON049556 | ON221789 | |
| | ZJUE 0167 | Quzhou, Zhejiang | Hybrid cv. Cocktail grapefruit | Twig melanose | ON035581 | ON221739 | ON113079 | ON049557 | ON221790 | |
| | ZJUE 0182 | Chun'an, Zhejiang | <i>C. maxima</i> | Twig dieback | ON035582 | ON221740 | ON113080 | ON049558 | ON221791 | |

Table 2 (cont.)

| Species ^a | Isolate | Location | Host | Associated symptoms | GenBank accession number ^b | | | | | |
|-------------------------|-----------------------|------------------------|-----------------------|---------------------------|---------------------------------------|----------|----------|----------|----------|----------|
| | | | | | ITS | cal | his3 | tef1 | tlb2 | |
| <i>D. eres</i> (cont.) | ZJUE 0248 | Changxingdao, Shanghai | Hybrid cv. Hongmeiren | Branch decay and gummosis | ON035563 | ON221721 | ON113061 | ON049539 | ON221772 | |
| | ZJUE 0267 | Jishou, Hunan | <i>C. unshiu</i> | Twig dieback | ON035583 | ON221741 | ON113081 | ON049559 | ON221792 | |
| | ZJUE 0298 | Nanfeng, Jiangxi | <i>C. reticulata</i> | Leaf melanose | ON035584 | ON221742 | ON113082 | ON049560 | ON221793 | |
| | ZJUE 0304 | Ruilu, Yunnan | <i>P. trifoliata</i> | Shoot blight | ON035585 | ON221743 | ON113083 | ON049561 | ON221794 | |
| | ZJUE 0316 | Wanzhou, Chongqing | <i>C. limon</i> | Aspiomatic twig | ON035586 | ON221744 | ON113084 | ON049562 | ON221795 | |
| | ZJUE 0317 | Fengjie, Chongqing | <i>C. reticulata</i> | Twig dieback | ON035587 | ON221745 | ON113085 | ON049563 | ON221796 | |
| | ZJUE 0360 | Congjiang, Guizhou | <i>C. reticulata</i> | Leaf melanose | OP218134 | OP265461 | OP265525 | OP265589 | OP265653 | |
| | ZJUE 0387 | Yizhang, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218135 | OP265462 | OP265526 | OP265590 | OP265654 | |
| | ZJUE 0389 | Yizhang, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218136 | OP265463 | OP265527 | OP265591 | OP265655 | |
| | ZJUE 0391 | Yizhang, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218137 | OP265464 | OP265528 | OP265592 | OP265656 | |
| | ZJUE 0395 | Yizhang, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218138 | OP265465 | OP265529 | OP265593 | OP265657 | |
| | ZJUE 0405 | Luxi, Hunan | <i>C. reticulata</i> | Leaf melanose | OP218139 | OP265466 | OP265530 | OP265594 | OP265658 | |
| | ZJUE 0422 | Shaoyang, Hunan | <i>C. unshiu</i> | Fruit melanose | OP218140 | OP265467 | OP265531 | OP265595 | OP265659 | |
| | <i>D. fulvicolor</i> | ZJUE 0310 | Tailong, Chongqing | <i>C. limon</i> | Twig dieback | ON035588 | ON221746 | ON113086 | ON049564 | ON221797 |
| ZJUE 0311 | | Tailong, Chongqing | <i>C. limon</i> | Twig dieback | ON035589 | ON221747 | ON113087 | ON049565 | ON221798 | |
| <i>D. fuscicola</i> | ZJUE 0371 | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218141 | OP265468 | OP265532 | OP265596 | OP265660 | |
| | ZJUE 0318 * | Fengjie, Chongqing | <i>C. reticulata</i> | Twig dieback | OR160316 | OR178783 | OR178799 | OR178815 | OR178831 | |
| <i>D. gammata</i> | ZJUE 0319 | Fengjie, Chongqing | <i>C. reticulata</i> | Twig dieback | OR160317 | OR178784 | OR178800 | OR178816 | OR178832 | |
| | ZJUE 0401 | Luxi, Hunan | <i>C. reticulata</i> | Leaf melanose | OR160318 | OR178785 | OR178801 | OR178817 | OR178833 | |
| <i>D. guangxiensis</i> | ZJUE 0424 | Ganzhou, Jiangxi | <i>C. sinensis</i> | Fruit melanose | OP218142 | OP265469 | OP265533 | OP265597 | OP265661 | |
| <i>D. hongkongensis</i> | ZJUE 0289 | Chenzhou, Hunan | <i>C. sinensis</i> | Twig dieback | ON035592 | ON221750 | ON113090 | ON049568 | ON221801 | |
| | ZJUE 0290 | Chenzhou, Hunan | <i>C. sinensis</i> | Twig dieback | ON035593 | ON221751 | ON113091 | ON049569 | ON221802 | |
| | ZJUE 0348 | Nanping, Fujian | <i>C. reticulata</i> | Fruit melanose | QQ703346 | QQ719550 | QQ719564 | QQ719578 | QQ719592 | |
| | ZJUE 0392 | Chenzhou, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218143 | OP265470 | OP265534 | OP265598 | OP265662 | |
| | ZJUE 0396 | Chenzhou, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218144 | OP265471 | OP265535 | OP265599 | OP265663 | |
| | ZJUE 0420 | Shaoyang, Hunan | <i>C. unshiu</i> | Leaf melanose | OP218145 | OP265472 | OP265536 | OP265600 | OP265664 | |
| | ZJUE 0427 | Yichun, Jiangxi | <i>C. reticulata</i> | Leaf melanose | OP218146 | OP265473 | OP265537 | OP265601 | OP265665 | |
| | ZJUE 0393 | Luxi, Hunan | <i>C. reticulata</i> | Leaf melanose | OP218147 | OP265474 | OP265538 | OP265602 | OP265666 | |
| | ZJUE 0400 | Chenzhou, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218148 | OP265475 | OP265539 | OP265603 | OP265667 | |
| | <i>D. jishouensis</i> | ZJUE 0265 * | Jishou, Hunan | <i>C. unshiu</i> | Twig dieback | OR160319 | OR178786 | OR178802 | OR178818 | OR178834 |
| ZJUE 0291 | | Jishou, Hunan | <i>C. unshiu</i> | Twig dieback | OR160320 | OR178787 | OR178803 | OR178819 | OR178835 | |
| ZJUE 0353 | | Zhangzhou, Fujian | <i>C. maxima</i> | Leaf melanose | OR160321 | OR178788 | OR178804 | OR178820 | OR178836 | |
| ZJUE 0356 | | Congjiang, Guizhou | <i>C. reticulata</i> | Leaf melanose | OR160322 | OR178789 | OR178805 | OR178821 | OR178837 | |
| ZJUE 0372 | | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OR160323 | OR178790 | OR178806 | OR178822 | OR178838 | |
| ZJUE 0374 | | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OR160324 | OR178791 | OR178807 | OR178823 | OR178839 | |
| ZJUE 0376 | | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OR160325 | OR178792 | OR178808 | OR178824 | OR178840 | |
| ZJUE 0379 | | Luxi, Hunan | <i>C. sinensis</i> | Fruit melanose | OR160326 | OR178793 | OR178809 | OR178825 | OR178841 | |
| ZJUE 0382 | | Luxi, Hunan | <i>C. sinensis</i> | Fruit melanose | OR160327 | OR178794 | OR178810 | OR178826 | OR178842 | |
| ZJUE 0421 | | Shaoyang, Hunan | <i>C. unshiu</i> | Fruit melanose | OR160328 | OR178795 | OR178811 | OR178827 | OR178843 | |
| ZJUE 0423 | | Ganzhou, Jiangxi | <i>C. sinensis</i> | Fruit melanose | OR160329 | OR178796 | OR178812 | OR178828 | OR178844 | |
| ZJUE 0263 | | Changxingdao, Shanghai | <i>C. unshiu</i> | Branch decay and gummosis | ON035594 | ON221752 | ON113092 | ON049570 | ON221803 | |
| ZJUE 0339 | | Meizhou, Guangdong | <i>C. maxima</i> | Leaf melanose | OR160298 | OR178886 | OR178889 | OR178892 | OR178895 | |
| ZJUE 0341 | | Meizhou, Guangdong | <i>C. maxima</i> | Leaf melanose | OR160299 | OR178887 | OR178890 | OR178893 | OR178896 | |
| ZJUE 0344 | Meizhou, Guangdong | <i>C. maxima</i> | Twig melanose | OR160300 | OR178888 | OR178891 | OR178894 | OR178897 | | |
| <i>D. novem</i> | ZJUE 0305 | Binchuan, Yunnan | Hybrid cv. Wogan | Twig dieback | ON035595 | ON221753 | ON113093 | ON049571 | ON221804 | |
| | ZJUE 0355 | Zhangzhou, Fujian | <i>C. maxima</i> | Leaf melanose | OP218149 | OP265476 | OP265540 | OP265604 | OP265668 | |

Table 2 (cont.)

| Species ^a | Isolate | Location | Host | Associated symptoms | GenBank accession number ^b | | | | |
|--------------------------|--------------------|------------------------|-------------------------|-----------------------|---------------------------------------|-----------------|-----------------|-----------------|-----------------|
| | | | | | ITS | cal | his3 | tef1 | tub2 |
| <i>D. penetrans</i> | ZJUE 0363 | Luxi, Hunan | <i>C. sinensis</i> | Fruit melanose | OQ703347 | OQ719551 | OQ719565 | OQ719579 | OQ719593 |
| | ZJUE 0394 | Chenzhou, Hunan | <i>C. sinensis</i> | Fruit melanose | OQ703348 | OQ719552 | OQ719566 | OQ719580 | OQ719594 |
| | ZJUE 0404 | Luxi, Hunan | <i>C. reticulata</i> | Fruit melanose | OP218150 | OP265477 | OP265541 | OP265605 | OP265669 |
| | ZJUE 0414 | Shaoyang, Hunan | <i>C. unshiu</i> | Leaf melanose | OQ703349 | OQ719553 | OQ719567 | OQ719581 | OQ719595 |
| | ZJUE 0345 | Nanping, Fujian | <i>C. reticulata</i> | Fruit melanose | OP218151 | OP265478 | OP265542 | OP265606 | OP265670 |
| <i>D. peticola</i> | ZJUE 0340 | Meizhou, Guangdong | <i>C. maxima</i> | Leaf melanose | OQ703350 | OQ719554 | OQ719568 | OQ719582 | OQ719596 |
| | ZJUE 0349 | Zhangzhou, Fujian | <i>C. maxima</i> | Leaf melanose | OQ703351 | OQ719555 | OQ719569 | OQ719583 | OQ719597 |
| | ZJUE 0350 | Zhangzhou, Fujian | <i>C. maxima</i> | Leaf melanose | OQ703352 | OQ719556 | OQ719570 | OQ719584 | OQ719598 |
| | ZJUE 0352 | Zhangzhou, Fujian | <i>C. maxima</i> | Leaf melanose | OQ703353 | OQ719557 | OQ719571 | OQ719585 | OQ719599 |
| | ZJUE 0307 * | Ruli, Yunan | <i>C. limon</i> | Twig dieback | OR160315 | OR178782 | OR178798 | OR178814 | OR178830 |
| <i>D. sackstonii</i> | ZJUE 0364 | Luxi, Hunan | <i>C. sinensis</i> | Fruit melanose | OP218152 | OP265479 | OP265543 | OP265607 | OP265671 |
| | ZJUE 0381 | Luxi, Hunan | <i>C. sinensis</i> | Fruit melanose | OP218153 | OP265480 | OP265544 | OP265608 | OP265672 |
| | ZJUE 0411 | Luxi, Hunan | <i>C. reticulata</i> | Fruit melanose | OP218121 | OP265448 | OP265512 | OP265576 | OP265640 |
| | ZJUE 0415 | Shaoyang, Hunan | <i>C. unshiu</i> | Fruit melanose | OP218154 | OP265481 | OP265545 | OP265609 | OP265673 |
| | ZJUE 0343 | Meizhou, Guangdong | <i>C. maxima</i> | Twig melanose | OP218155 | OP265482 | OP265546 | OP265610 | OP265674 |
| <i>D. sexualispora</i> | ZJUE 0418 * | Shaoyang, Hunan | <i>C. unshiu</i> | Fruit melanose | OR160330 | OR178797 | OR178813 | OR178829 | OR178845 |
| | ZJUE 0163 | Quzhou, Zhejiang | <i>C. paradisii</i> | Leaf necrosis | ON221754 | ON113094 | ON113094 | ON049572 | ON221805 |
| | ZJUE 0278 | Jishou, Hunan | <i>C. reticulata</i> | Twig dieback | ON035597 | ON221755 | ON113095 | ON049573 | ON221806 |
| | ZJUE 0361 | Chenzhou, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218156 | OP265483 | OP265547 | OP265611 | OP265675 |
| | ZJUE 0386 | Chenzhou, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218157 | OP265484 | OP265548 | OP265612 | OP265676 |
| <i>D. spinosa</i> | ZJUE 0419 | Shaoyang, Hunan | <i>C. unshiu</i> | Fruit melanose | OP218158 | OP265485 | OP265549 | OP265613 | OP265677 |
| | ZJUE 0434 | Taizhou, Zhejiang | <i>C. unshiu</i> | Fruit melanose | OP218159 | OP265486 | OP265550 | OP265614 | OP265678 |
| | ZJUE 0354 | Zhangzhou, Fujian | <i>C. maxima</i> | Leaf melanose | OP218160 | OP265487 | OP265551 | OP265615 | OP265679 |
| | ZJUE 0399 | Luxi, Hunan | <i>C. reticulata</i> | Leaf melanose | OP218161 | OP265488 | OP265552 | OP265616 | OP265680 |
| | ZJUE 0366 | Luxi, Hunan | <i>C. sinensis</i> | Fruit melanose | OQ703354 | OQ719558 | OQ719572 | OQ719586 | OQ719600 |
| <i>D. tectonae</i> | ZJUE 0368 | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OQ703355 | OQ719559 | OQ719573 | OQ719587 | OQ719601 |
| | ZJUE 0373 | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OQ703356 | OQ719560 | OQ719574 | OQ719588 | OQ719602 |
| | ZJUE 0378 | Luxi, Hunan | <i>C. sinensis</i> | Fruit melanose | OP218162 | OP265489 | OP265553 | OP265617 | OP265681 |
| | ZJUE 0390 | Chenzhou, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218163 | OP265490 | OP265554 | OP265618 | OP265682 |
| | ZJUE 0302 | Ruli, Yunan | <i>P. trifoliata</i> | Shoot blight | ON035598 | ON221756 | ON113096 | ON049574 | ON221807 |
| <i>D. unshiuensis</i> | ZJUE 0303 | Ruli, Yunan | <i>P. trifoliata</i> | Shoot blight | ON035599 | ON221757 | ON113097 | ON049575 | ON221808 |
| | ZJUE 0347 | Nanping, Fujian | <i>C. reticulata</i> | Fruit melanose | OP218164 | OP265491 | OP265555 | OP265619 | OP265683 |
| | ZJUE 0432 | Taizhou, Zhejiang | <i>C. reticulata</i> | Fruit melanose | OP218165 | OP265492 | OP265556 | OP265620 | OP265684 |
| | ZJUE 0129 | Linshui, Zhejiang | <i>C. unshiu</i> | Twig dieback | ON035600 | ON221758 | ON113098 | ON049576 | ON221809 |
| | ZJUE 0177 | Lishui, Zhejiang | <i>C. sinensis</i> | Twig dieback | ON035601 | ON221759 | ON113099 | ON049577 | ON221810 |
| <i>D. velutina</i> | ZJUE 0178 | Lishui, Zhejiang | <i>C. sinensis</i> | Twig dieback | ON035602 | ON221760 | ON113100 | ON049578 | ON221811 |
| | ZJUE 0181 | Lishui, Zhejiang | <i>C. sinensis</i> | Twig dieback | ON035603 | ON221761 | ON113101 | ON049579 | ON221812 |
| | ZJUE 0370 | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218166 | OP265493 | OP265557 | OP265621 | OP265685 |
| | ZJUE 0385 | Luxi, Hunan | <i>C. sinensis</i> | Leaf melanose | OP218167 | OP265494 | OP265558 | OP265622 | OP265686 |
| | ZJUE 0430 | Yichun, Jiangxi | <i>C. reticulata</i> | Leaf melanose | OP218168 | OP265495 | OP265559 | OP265623 | OP265687 |
| <i>D. xishuangbanica</i> | ZJUE 0342 | Meizhou, Guangdong | <i>C. maxima</i> | Leaf melanose | OP218169 | OP265496 | OP265560 | OP265624 | OP265688 |

^a Species names in bold represent new species described in this study.^b ITS, internal transcribed spacer region and intervening 5.8S rDNA gene; cal, calmodulin; his3, histone H3; tef1, translation elongation factor 1-alpha; tub2, beta-tubulin.

* Isolates represent ex-type.

Table 3 Isolates from other studies used in the phylogenetic analyses.

| Species | Culture no. ^a | Host | Location | Collector | ITS | cal | his3 | tefl | tub2 |
|---------------------------------|--------------------------------------|-------------------------------|----------------|-------------------|----------|----------|----------|----------|----------|
| <i>D. acaciaram</i> | CBS138862 * | <i>Vachellia tortilis</i> | Tanzania | M.J. Wingfield | KP004460 | — | KP004504 | — | KP004509 |
| <i>D. acericola</i> | MFLUCC 17-0956 * | <i>Acer negundo</i> | Italy | — | KY964224 | KY964137 | — | KY964180 | KY964074 |
| <i>D. acerigena</i> | CFCC 52554 * | <i>Acer tataricum</i> | China | Q. Yang | MH121489 | MH121413 | MH121449 | MH121531 | — |
| <i>D. acuta</i> | PSCG 047 * | <i>Pyrus pyrifolia</i> | China | Q. Bai | MK626957 | MK691125 | MK726161 | MK654802 | MK691225 |
| | PSCG 046 | <i>Pyrus pyrifolia</i> | China | Q. Bai | MK626958 | MK691125 | MK726162 | MK654803 | MK691224 |
| <i>D. alangii</i> | CFCC 52556 * | <i>Alangium kurzii</i> | China | Q. Yang | MH121491 | MH121415 | MH121451 | MH121533 | MH121573 |
| | CFCC 52557 | <i>Alangium kurzii</i> | China | Q. Yang | MH121492 | MH121416 | MH121452 | MH121534 | MH121574 |
| <i>D. albosinensis</i> | CFCC 53066 * | <i>Betula albosinensis</i> | China | N. Jiang | MK432659 | MK442979 | MK443004 | MK578133 | MK578059 |
| <i>D. alleghaniensis</i> | CBS 495.72 = ATCC 24097 * | <i>Betula alleghaniensis</i> | Canada | R.H. Arnold | KC343007 | KC343249 | KC343491 | KC343733 | KC343975 |
| <i>D. alinea</i> | CBS 146.46 * | <i>Alnus</i> sp. | Netherlands | S. Truter | KC343008 | KC343250 | KC34349 | KC343734 | KC343976 |
| <i>D. arpelina</i> | CBS 114016 * | <i>Vitis vinifera</i> | France | — | AF230751 | JX197443 | — | AY745056 | JX275452 |
| <i>D. amygdali</i> | CBS 126679 * | <i>Prunus dulcis</i> | Portugal | E. Diogo | KC343022 | KC343264 | KC343506 | KC343748 | KC343990 |
| <i>D. anacardii</i> | CBS 720.97 * | <i>Anacardium occidentale</i> | East Africa | M. Puccioni | KC343024 | KC343266 | KC343508 | KC343750 | KC343992 |
| | CBS 144610 = CPC 33074 | Unidentified leaf litter | South Africa | P.W. Crous | MK442578 | MK442651 | — | MK442692 | — |
| <i>D. apiculata</i> | CGMCC 3.17533 * | <i>Camellia sinensis</i> | China | Y. Zhang | KP267896 | — | — | KP267970 | KP293476 |
| | LC3364 | <i>Camellia sinensis</i> | China | Y. Zhang | KP267897 | — | — | KP267961 | KP293467 |
| <i>D. arecae</i> | CBS 161.64 * | <i>Areca catechu</i> | India | H.C. Srivastava | KC343032 | KC343274 | KC343516 | KC343758 | KC344000 |
| | ZJUD55 | <i>Citrus grandis</i> | China | X. Hou | KJ490590 | — | KJ490532 | KJ490469 | KJ490411 |
| | ZJUD65 | <i>Citrus sinensis</i> | China | F. Huang | KJ490600 | — | KJ490542 | KJ490479 | KJ490421 |
| <i>D. arengae</i> | CBS 114979 * | <i>Arenga engleri</i> | China | K.D. Hyde | KC343034 | KC343276 | KC343518 | KC343760 | KC344002 |
| <i>D. aseana</i> | MFLUCC 12-0299a * | Unknown dead leaf | Thailand | N. Tangthirasunun | KT459414 | KT459464 | — | KT459448 | KT459432 |
| <i>D. beilharziae</i> | BRIP 54792 * | <i>Indigofera australis</i> | Australia | V.C. Beilharz | JX862529 | — | — | JX862535 | KF170921 |
| <i>D. betulae</i> | CFCC 50469 * | <i>Betula platyphylla</i> | China | X.L. Fan | KT732950 | KT732997 | — | KT733016 | KT733020 |
| <i>D. betulicola</i> | CFCC 51128 * | <i>Betula albosinensis</i> | China | X.L. Fan & Z. Du | KX024653 | KX024659 | KX024661 | KX024655 | KX024657 |
| <i>D. betulina</i> | CFCC 52560 * | <i>Betula albosinensis</i> | China | Q. Yang | MH121495 | MH121419 | MH121455 | MH121537 | MH121577 |
| <i>D. biconicta</i> | CBS 121004 * | <i>Juglans</i> sp. | USA | L. Vasilyeva | KC343134 | KC343376 | KC343618 | KC343860 | KC344102 |
| <i>D. biconispora</i> | CGMCC 3.17252 = ZJUD62 * | <i>Citrus grandis</i> | China | F. Huang | KJ490597 | MT898460 | KJ490539 | KJ490476 | KJ490418 |
| | YJ1 | <i>Sapindus mukorossi</i> | China | — | MN901242 | MT459786 | MT113117 | MT113118 | MT113116 |
| <i>D. biguttulata</i> | CGMCC 3.17248 = ZJUD47 * | <i>Citrus limon</i> | China | F. Huang | KJ490582 | — | KJ490524 | KJ490461 | KJ490403 |
| | ZJUD48 | <i>Citrus limon</i> | China | F. Huang | KJ490583 | — | KJ490525 | KJ490462 | KJ490404 |
| <i>D. biguttusis</i> | CGMCC 3.17081 * | <i>Lithocarpus glabra</i> | China | W. Sun | KF576282 | — | — | KF576257 | KF576306 |
| <i>D. bohemiae</i> | CBS 143347 = CPC 28222 * | <i>Vitis vinifera</i> | Czech Republic | — | MG281015 | MG281710 | MG281361 | MG281536 | MG281188 |
| | CBS 143348 | <i>Vitis vinifera</i> | Czech Republic | — | MG281016 | MG281711 | MG281362 | MG281537 | MG281189 |
| <i>D. camptothecicola</i> | CFCC 51632 | <i>Camptotheca acuminata</i> | China | Q. Yang | KY203726 | KY228877 | KY228881 | KY228887 | KY228893 |
| <i>D. caryae</i> | CFCC 52563 * | <i>Carya ilinoensis</i> | China | Q. Yang | MH121498 | MH121422 | MH121458 | MH121540 | MH121580 |
| | PSCG 520 | <i>Pyrus pyrifolia</i> | China | Q. Yang | MK626952 | MK691200 | MK726202 | MK654895 | MK691315 |
| <i>D. castaneae-mollissimae</i> | DNP 128 * | <i>Castanea mollissima</i> | China | S.X. Jiang | JF957786 | JX197430 | — | JX275401 | JX275438 |
| | PSCG 520 | <i>Pyrus pyrifolia</i> | China | Q. Yang | MK626952 | MK691200 | MK726202 | MK654895 | MK691315 |
| <i>D. celastri</i> | CBS 139.27 * | <i>Celastrus</i> sp. | USA | L.E. Wehmeyer | KC343047 | KC343289 | KC343531 | KC343773 | KC344015 |
| <i>D. celeris</i> | CBS 143349 = CPC 28262 * | <i>Vitis vinifera</i> | Czech Republic | — | MG281017 | MG281712 | MG281363 | MG281538 | MG281190 |
| <i>D. ceridis</i> | CFCC 52565 * | <i>Cercis chinensis</i> | China | Q. Yang | MH121500 | MH121424 | MH121460 | MH121542 | MH121582 |
| | CFCC 52566 | <i>Cercis chinensis</i> | China | Q. Yang | MH121501 | MH121425 | MH121461 | MH121543 | MH121583 |
| <i>D. chensiensis</i> | CFCC 52567 * | <i>Abies chensiensis</i> | China | Q. Yang | MH121502 | MH121426 | MH121462 | MH121544 | MH121584 |
| <i>D. cinnamomi</i> | CFCC 52569 * | <i>Cinnamomum</i> sp. | China | Q. Yang | MH121504 | — | MH121464 | MH121546 | MH121586 |
| <i>D. citri</i> | CBS 135422 * | <i>Citrus</i> sp. | USA | L. Timmer | KC843311 | — | — | KC843071 | KC843187 |
| | ZJUD1 | <i>Citrus reticulata</i> | China | F. Huang | JQ954654 | — | KJ490514 | JQ954671 | KJ490395 |
| <i>D. citriasiana</i> | CBS134240 = CGMCC3.15224 = ZJUD30 * | <i>Citrus unshiu</i> | China | F. Huang | JQ954645 | KC357491 | KJ490515 | JQ954663 | KC357459 |
| | ZJUD82 | <i>Citrus maxima</i> | China | F. Huang | KJ490617 | — | KJ490559 | KJ490496 | KJ490438 |
| <i>D. citrichinensis</i> | CBS 134242 = CGMCC3.15225 = ZJUD34 * | <i>Citrus unshiu</i> | China | F. Huang | JQ954648 | KC357494 | KJ490516 | JQ954666 | KJ490396 |

Table 3 (cont.)

| Species | Culture no. ^a | Host | Location | Collector | ITS | cal | his3 | tef1 | tub2 |
|---------------------------------|---------------------------------------|---------------------------------|--------------|-----------------|----------|----------|----------|----------|----------|
| <i>D. collariana</i> | MFLUCC 17-2636 * | <i>Magnolia champaca</i> | Thailand | – | MG806115 | MG783042 | – | MG783040 | MG783041 |
| <i>D. compacta</i> | CGMCC 3.17536 = LC3083 * | <i>Camellia sinensis</i> | China | F. Liu | KP267854 | – | KP293508 | KP267928 | KP293434 |
| | LC3084 | <i>Camellia sinensis</i> | China | F. Liu | KP267855 | – | KP293509 | KP267929 | KP293435 |
| <i>D. conica</i> | CFCC 52571 * | <i>Alangium chinense</i> | China | Q. Yang | MH121506 | MH121428 | MH121466 | MH121548 | MH121588 |
| <i>D. convolvuli</i> | CBS 124654 = DP0727 * | <i>Convolvulus arvensis</i> | Turkey | D. Berner | KC343054 | KC343296 | KC343538 | KC343780 | KC344022 |
| <i>D. coryli</i> | CFCC 53083 * | <i>Corylus mandshurica</i> | China | N. Jiang | MK442661 | MK442981 | MK443006 | MK578135 | MK578061 |
| | CFCC 53084 | <i>Corylus mandshurica</i> | China | N. Jiang | MK442662 | MK442982 | MK443007 | MK578136 | MK578062 |
| <i>D. cotoneastri</i> | CBS 439.82 * | <i>Cotoneaster</i> sp. | UK | H. Butin | FJ889450 | JX197429 | – | GQ250341 | JX275437 |
| <i>D. diospyricola</i> | CBS 136552 = CPC 21169 * | <i>Diospyros whyteana</i> | South Africa | P.W. Crous | KF777156 | – | – | – | – |
| <i>D. discoidispora</i> | CGMCC 3.17255 = ICMP 20662 = ZJUD89 * | <i>Citrus unshiu</i> | China | F. Huang | KJ490624 | – | KJ490566 | KJ490503 | KJ490445 |
| <i>D. ellipicola</i> | CGMCC 3.17084 * | <i>Lithocarpus glabra</i> | China | W. Sun | KF576270 | – | KF576245 | KF576294 | KF576294 |
| <i>D. endophytica</i> | CBS 133811 = LGMF916 * | <i>Schinus terebinthifolius</i> | Brazil | J. Lima | KC343065 | KC343307 | KC343549 | KC343791 | KC344033 |
| <i>D. eres</i> | AR5193 * | <i>Ulmus</i> sp. | Germany | R. Schumacher | KJ210529 | KJ434999 | KJ420850 | KJ210550 | KJ420799 |
| | CBS 101742 | <i>Fraxinus</i> sp. | Netherlands | G.J.M. Verkley | KC343073 | KC343315 | KC343557 | KC343799 | KC344041 |
| | DLR12a | <i>Vitis vinifera</i> | France | L. Philippe | KJ210518 | KJ434996 | KJ420833 | KJ210542 | KJ420783 |
| | DP0438 | <i>Ulmus minor</i> | Netherlands | W. Jaklitsch | KJ210532 | KJ435016 | KJ420886 | KJ210553 | KJ420816 |
| | FAU506 | <i>Cornus florida</i> | USA | F. Uecker | KJ210526 | KJ435012 | KJ420842 | JO807403 | KJ420792 |
| <i>D. eugeniae</i> | CBS 444.82 * | <i>Eugenia aromatica</i> | Indonesia | R. Kasim | KC343098 | KC343340 | KC343582 | KC343824 | KC344066 |
| <i>D. fraxini-angustifoliae</i> | BRIP 54781 * | <i>Fraxinus angustifolia</i> | Australia | L. Smith | JX862528 | – | – | JX862534 | KF170920 |
| <i>D. fraxinicola</i> | CFCC 52582 * | <i>Fraxinus chinensis</i> | China | Q. Yang | MH121517 | MH121435 | – | MH121559 | – |
| <i>D. fukushii</i> | MAFF 625034 | <i>Pyrus pyrifolia</i> | Japan | – | JO807469 | – | – | JO807418 | – |
| <i>D. fulvicolor</i> | PSCG 051 * | <i>Pyrus pyrifolia</i> | China | Q. Bai | MK628859 | MK691132 | MK726163 | MK654806 | MK691236 |
| | PSCG 057 | <i>Pyrus pyrifolia</i> | China | Q. Bai | MK628858 | MK691131 | MK726164 | MK654810 | MK691233 |
| | CGMCC 3.17087 * | <i>Lithocarpus glabra</i> | China | W. Sun | KF576281 | KF576233 | – | KF576256 | KF576305 |
| <i>D. fuscicola</i> | PSCG 015 | <i>Pyrus pyrifolia</i> | China | – | MK626911 | MK691210 | – | MK654861 | MK691320 |
| <i>D. ganjae</i> | CBS 180.91 * | <i>Cannabis sativa</i> | USA | J.M. McPartland | KC343112 | KC343354 | KC343596 | KC343838 | KC344080 |
| <i>D. guangxiensis</i> | JZB320094 * | <i>Vitis vinifera</i> | China | – | MK335772 | MK736727 | – | MK523566 | MK500168 |
| | JZB320093 | <i>Vitis vinifera</i> | China | – | MK335771 | MK736726 | – | MK523565 | MK500167 |
| <i>D. guylae</i> | BRIP 54025 * | <i>Helianthus annuus</i> | Australia | – | JF431299 | – | – | JN645803 | – |
| <i>D. helicis</i> | CBS 138596 = AR5211 * | <i>Hedera helix</i> | France | R. Schumacher | KJ210538 | KJ435043 | KJ420875 | KJ210559 | KJ420828 |
| <i>D. heliconiae</i> | SAUCC194.77 * | <i>Heliconia metallica</i> | China | S.T. Huang | MT822605 | MT855684 | MT855573 | MT855917 | MT855802 |
| | SAUCC194.75 | <i>Heliconia metallica</i> | China | S.T. Huang | MT822603 | MT855682 | MT855571 | MT855915 | MT855800 |
| <i>D. heterophyllae</i> | CBS 143769 = CPC26215 * | <i>Acacia heterophylla</i> | France | – | MG600222 | MG600218 | MG600224 | MG600224 | MG600226 |
| <i>D. hispaniae</i> | CBS 143351 = CPC 30321 * | <i>Vitis vinifera</i> | Spain | – | MG281123 | MG281820 | MG281471 | MG281644 | MG281296 |
| <i>D. hongkongensis</i> | CBS 115448 * | <i>Dichroa febrifuga</i> | China | K.D. Hyde | KC343119 | KC343361 | – | KC343845 | KC344087 |
| <i>D. hubeiensis</i> | JZB320123 * | <i>Vitis vinifera</i> | China | X.H. Li | MK335809 | MK500235 | – | MK523570 | MK500148 |
| | JZB320122 | <i>Vitis vinifera</i> | China | X.H. Li | MK335808 | MK500234 | – | MK523569 | MK500147 |
| <i>D. incompleta</i> | CGMCC 3.18288 * | <i>Camellia sinensis</i> | China | F. Liu | KX987794 | KX999289 | KX999265 | KX999186 | KX999226 |
| <i>D. inconspicua</i> | CBS 133813 * | <i>Maytenis ilicifolia</i> | China | Q. Yang | KC343123 | KC343365 | KC343607 | KC343849 | KC344091 |
| <i>D. infecunda</i> | CBS 133812 = CPC 20282 = LGMF906 * | <i>Schinus terebinthifolius</i> | Brazil | J. Lima | KC343126 | KC343368 | – | KC343852 | KC344094 |
| <i>D. isoberliniae</i> | CPC 22549 * | <i>Isobertinia angolensis</i> | Zambia | M. van der Bank | KJ869133 | – | – | – | KJ869245 |
| <i>D. juglandicola</i> | CFCC 51134 * | <i>Juglans mandshurica</i> | China | Q. Yang | KJ985101 | KX024616 | KX024622 | KX024628 | KX024634 |
| <i>D. kadsurae</i> | CFCC 52586 * | <i>Kadsura longipedunculata</i> | China | Q. Yang | MH121521 | MH121439 | – | MH121563 | MH121600 |
| <i>D. kochmanii</i> | BRIP 54033 * | <i>Helianthus annuus</i> | Australia | S.M. Thompson | JF431295 | – | – | JN645809 | – |
| <i>D. kongii</i> | BRIP 54031 * | <i>Helianthus annuus</i> | Australia | S.M. Thompson | JF431301 | – | – | JN645797 | – |
| <i>D. limonicola</i> | CBS 142549 = CPC 28200 * | <i>Citrus limon</i> | Malta | – | MF418422 | MF418256 | MF418342 | MF418501 | MF418582 |
| | CBS 142550 = CPC 31137 | <i>Citrus limon</i> | Malta | – | MF418423 | MF418257 | MF418343 | MF418502 | MF418583 |
| <i>D. litchicola</i> | BRIP 54900 * | <i>Litchi chinensis</i> | Australia | K.R.E. Grice | JX862553 | – | – | JX862539 | KF170925 |
| <i>D. litchii</i> | SAUCC194.22 * | <i>Litchi chinensis</i> | China | S.T. Huang | MT822550 | MT855635 | MT855519 | MT855863 | MT855747 |

Table 3 (cont.)

| Species | Culture no. ^a | Host | Location | Collector | ITS | cal | his3 | tef1 | tub2 |
|--------------------------------|---------------------------------------|--------------------------------|--------------------|--------------------------|----------|----------|----------|----------|----------|
| <i>D. lithocarpus</i> | CGMCC 3.15175 * | <i>Lithocarpus glabra</i> | China | W. Sun | KC153104 | KF576235 | — | KC153095 | KF576311 |
| | CGMCC 3.17098 | <i>Lithocarpus glabra</i> | China | W. Sun | KF576276 | KF576228 | — | KF576251 | KF576300 |
| <i>D. longicicola</i> | CGMCC 3.17089 * | <i>Lithocarpus glabra</i> | China | W. Sun | KF576267 | — | — | KF576242 | KF576291 |
| <i>D. loniceræ</i> | FAU599 = ATCC 60325 * | <i>Glycine max</i> | USA | T.W. Hobbs | KJ590728 | KJ612124 | KJ659188 | KJ590767 | KJ610883 |
| <i>D. machili</i> | MFLUCC 17-0963 * | <i>Lonicera</i> sp. | Italy | E. Camporesi | KY964190 | KY964116 | — | KY964146 | KY964073 |
| | SAUCC 194.111 * | <i>Machilia pingii</i> | China | S.T. Huang | MT822639 | MT855718 | MT855606 | MT855951 | MT855836 |
| | SAUCC194.69 | <i>Pometia pinnata</i> | China | S.T. Huang | MT822597 | MT855677 | MT855565 | MT855909 | MT855794 |
| <i>D. mahothocarpus</i> | CGMCC 3.15181 * | <i>Lithocarpus glabra</i> | China | W. Sun | KC153096 | KT459461 | — | KC153087 | KF576312 |
| <i>D. maritima</i> | NB365-71 * | <i>Picea rubens</i> | Canada | J.B. Tanney | KU552025 | MN136126 | MN136175 | KU552023 | KU574615 |
| <i>D. masirevicii</i> | BRIP 57892a * | <i>Helianthus annuus</i> | Australia | — | KJ197276 | — | — | KJ197239 | KJ197257 |
| <i>D. mellitensis</i> | CBS 142551 = CPC 27873 * | <i>Citrus limon</i> | Malta | V. Guarnaccia | MF418424 | MF418258 | MF418344 | MF418504 | MF418584 |
| | CPC 27875 | <i>Citrus limon</i> | Malta | V. Guarnaccia | MF418425 | MF418259 | MF418345 | MF418503 | MF418585 |
| <i>D. middletonii</i> | BRIP 54884e * | <i>Rapistrum rugostrum</i> | Australia | — | KJ197286 | — | — | KJ197248 | KJ197266 |
| <i>D. miricæ</i> | BRIP 54736j * | <i>Helianthus annuus</i> | Australia | — | KJ197282 | — | — | KJ197244 | KJ197262 |
| <i>D. momicola</i> | MFLUCC 16-0113 * | <i>Prunus persica</i> | China | X.H. Li | KU557563 | KU557611 | — | KU557631 | KU557587 |
| | MFLUCC 16-0113 * | <i>Prunus persica</i> | China | X.H. Li | KU557563 | KU557611 | — | KU557631 | KU557587 |
| | MFLUCC 16-0113 * | <i>Prunus persica</i> | China | X.H. Li | KU557563 | KU557611 | — | KU557631 | KU557587 |
| <i>D. multiguttulata</i> | CGMCC 3.17258 = ICMP 20656 = ZJUD98 * | <i>Citrus maxima</i> | China | F. Huang | KJ490633 | — | KJ490575 | KJ490512 | KJ490454 |
| <i>D. musigena</i> | CBS 129519 = CPC 17026 * | <i>Musa</i> sp. | Australia | P.W. Crous & R.G. Shivas | KC343143 | KC343385 | KC343627 | KC343869 | KC344111 |
| <i>D. neilliae</i> | CBS 144.27 * | <i>Spiraea</i> sp. | USA | L.E. Wehmeyer | KC343144 | KC343386 | KC343628 | KC343870 | KC344112 |
| <i>D. nobilis</i> | CBS 200.39 | <i>Laurus nobilis</i> | Germany | Kotthoff | KC343151 | KC343393 | KC343635 | KC343877 | KC344119 |
| <i>D. novem</i> | CBS 127270 * | <i>Glycine max</i> | Croatia | T. Duvnjak | KC343156 | KC343398 | KC343640 | KC343882 | KC344124 |
| | CBS 127271 | <i>Glycine max</i> | Croatia | T. Duvnjak | KC343157 | KC343399 | KC343641 | KC343883 | KC344125 |
| <i>D. oncostoma</i> | CBS 589.78 | <i>Robinia pseudoacacia</i> | France | H.A. van der Aa | KC343162 | KC343404 | KC343646 | KC343888 | KC344130 |
| <i>D. oracchini</i> | CGMCC 3.17531 = LC3166 * | <i>Camellia sinensis</i> | China | F. Liu | KP267863 | — | KP293517 | KP267937 | KP293443 |
| <i>D. ovalispora</i> | CGMCC 3.17256 = ICMP 20659 = ZJUD98 * | <i>Citrus limon</i> | China | F. Huang | KJ490628 | — | KJ490570 | KJ490507 | KJ490449 |
| <i>D. padina</i> | CFCC 52590 * | <i>Persea americana</i> | China | Q. Yang | MH121525 | MH121443 | MH121483 | MH121567 | MH121604 |
| <i>D. pascoei</i> | BRIP 54847 * | <i>Persea americana</i> | Australia | — | JX862532 | — | — | JX862538 | KF170924 |
| <i>D. passifloricola</i> | CBS 141329 = CPC 27480 * | <i>Passiflora foetida</i> | Malaysia | M.J. Wingfield | KX228292 | — | KX228367 | — | KX228387 |
| <i>D. penitriteum</i> | CGMCC 3.17532 = LC3353 * | <i>Camellia sinensis</i> | China | F. Liu | KP714505 | — | KP714493 | KP714517 | KP714529 |
| <i>D. perseæ</i> | CBS 151.73 * | <i>Persea gratissima</i> | Netherlands | E. Laville | KC343173 | KC343415 | KC343657 | KC343899 | KC344141 |
| <i>D. pescicola</i> | MFLUCC 16-0105 * | <i>Prunus persica</i> | China | X.H. Li | KU557555 | KU557603 | — | KU557623 | KU557579 |
| | MFLUCC 16-0106 | <i>Prunus persica</i> | China | X.H. Li | KU557556 | KU557604 | — | KU557624 | KU557580 |
| <i>D. phragmitis</i> | CBS 138897 * | <i>Phragmites australis</i> | China | P.W. Crous & Y. Zhang | KP004445 | — | KP004503 | — | KP004507 |
| <i>D. phaseolorum</i> | CBS 116019 = STAM 30 | <i>Caperonia palustris</i> | USA | A. Mengistu | KC343175 | KC343417 | KC343659 | KC343901 | KC344143 |
| | CBS 116020 = STAM 31 | <i>Aster exilis</i> | USA | A. Mengistu | KC343176 | KC343418 | KC343660 | KC343902 | KC344144 |
| <i>D. podocarpimacrophylli</i> | CGMCC 3.18281 = LC 6155 * | <i>Podocarpus macrophyllus</i> | China | W.J. Duan | KX998774 | KX999278 | KX999246 | KX999167 | KX999207 |
| <i>D. pometiae</i> | SAUCC 194.72 * | <i>Pometia pinnata</i> | China | S.T. Huang | MT822600 | MT855679 | MT855568 | MT855912 | MT855797 |
| | SAUCC 194.19 | <i>Persea americana</i> | China | S.T. Huang | MT822547 | MT855632 | MT855516 | MT855861 | MT855744 |
| <i>D. pseudomangiferae</i> | CBS 101339 * | <i>Mangifera indica</i> | Dominican Republic | P. de Leeuw | KC343181 | KC343423 | KC343665 | KC343907 | KC344149 |
| <i>D. pseudophoenicicola</i> | CBS 462.69 * | <i>Phoenix dactylifera</i> | Spain | H.A. van der Aa | KC343184 | KC343426 | KC343668 | KC343910 | KC344152 |
| <i>D. pterocarpicola</i> | MFLUCC 10-0571 * | <i>Pterocarpus indicus</i> | Thailand | — | JQ619899 | JX197451 | — | JX275416 | JX275460 |
| <i>D. pterocarpicola</i> | MFLUCC 10-0580a * | <i>Pterocarpus indicus</i> | Thailand | — | JQ619887 | JX197433 | — | JX275403 | JX275441 |
| <i>D. pulla</i> | CBS 338.89 * | <i>Hedera helix</i> | Yugoslavia | M. Muntanola-Cvetkovic | KC343152 | KC343394 | KC343636 | KC343878 | KC344120 |
| <i>D. pyracanthae</i> | CAA 483 * | <i>Pyracantha coccinea</i> | Portugal | — | KY435635 | KY435656 | KY435645 | KY435625 | KY435666 |
| <i>D. roseae</i> | MFLUCC 17-2658 * | <i>Rosa</i> sp. | Thailand | E.B.G. Jones | MG828894 | — | — | — | MG843878 |
| | MFLUCC 17-2574 | <i>Sennea siamea</i> | Thailand | R.H. Perera | MG906793 | — | — | — | MG968954 |
| <i>D. rosicola</i> | MFLU 17-0646 * | <i>Rosa</i> sp. | UK | E.B.G. Jones | MG828895 | — | — | — | MG843877 |
| <i>D. rostrata</i> | CFCC 50062 * | <i>Juglans mandshurica</i> | China | X.L. Fan | KP208847 | KP208849 | KP208851 | KP208853 | KP208855 |

Table 3 (cont.)

| Species | Culture no. ^a | Host | Location | Collector | ITS | cal | his3 | tef1 | tub2 |
|-----------------------------|---------------------------------------|------------------------------|-------------------|-----------------|----------|----------|----------|----------|----------|
| <i>D. saccharata</i> | CBS 116311 * | <i>Protea repens</i> | South Africa | S. Denman | KC343190 | KC343432 | KC343674 | KC343916 | KC344158 |
| <i>D. sackstonii</i> | BRIP 54669b * | <i>Helianthus annuus</i> | Australia | – | KJ197287 | – | – | KJ197249 | KJ197267 |
| <i>D. sambucusii</i> | CFCC 51986 * | <i>Sambucus williamsii</i> | China | Q. Yang | KY852495 | KY852499 | KY852503 | KY852507 | KY852511 |
| | CFCC 51987 * | <i>Sambucus williamsii</i> | China | Q. Yang | KY852496 | KY852500 | KY852504 | KY852508 | KY852512 |
| <i>D. schisandrae</i> | CFCC 51988 * | <i>Schisandra chinensis</i> | China | Q. Yang | KY852497 | KY852501 | KY852505 | KY852509 | KY852513 |
| <i>D. schoeni</i> | MFLU 15-1279 * | <i>Schoenus nigricans</i> | Italy | – | KY964226 | KY964139 | – | KY964182 | KY964109 |
| <i>D. sennae</i> | CFCC 51636 * | <i>Senna bicapsularis</i> | China | – | KY203724 | KY228875 | KY228879 | KY228885 | KY228891 |
| <i>D. sennicola</i> | CFCC 51634 * | <i>Senna bicapsularis</i> | China | Q. Yang | KY203722 | KY228873 | – | KY228883 | KY228889 |
| | CFCC 51635 * | <i>Senna bicapsularis</i> | China | Q. Yang | KY203723 | KY228874 | KY228880 | KY228884 | KY228890 |
| <i>D. serafiniae</i> | BRIP 55665a * | <i>Helianthus annuus</i> | Australia | – | KJ197274 | – | – | KJ197236 | KJ197254 |
| <i>D. shaanxiensis</i> | CFCC 53106 * | On branches of liana | China | N. Jiang | MK432654 | MK442976 | MK443001 | MK578130 | – |
| | CFCC 53107 * | On branches of liana | China | N. Jiang | MK432655 | MK442977 | MK443002 | MK578133 | – |
| <i>D. siamensis</i> | MFLUCC 10-573a * | <i>Dasymaschalos</i> sp. | Thailand | – | JQ619879 | – | – | JX275393 | JX275429 |
| <i>D. sojae</i> | FAU 635 * | <i>Glycine max</i> | USA | – | KJ590719 | KJ612116 | KJ659208 | KJ590762 | KJ610875 |
| <i>D. spinosa</i> | PSCG 383 * | <i>Pyrus pyrifolia</i> | China | Y.S. Guo | MK626849 | MK691129 | MK726156 | MK654811 | MK691234 |
| <i>D. sterilis</i> | CBS 136969 * | <i>Vaccinium corymbosum</i> | Italy | – | KJ160579 | KJ160548 | MF418350 | KJ160611 | KJ160528 |
| | CBS 136970 * | <i>Vaccinium corymbosum</i> | Italy | – | KJ160580 | KJ160549 | – | KJ160612 | KJ160529 |
| <i>D. subclavata</i> | CGMCC 3.17257 = ICMP 20663 = ZJUD95 * | <i>Citrus unshiu</i> | China | X. Hou | KJ490630 | – | KJ490572 | KJ490509 | KJ490451 |
| <i>D. subellipicola</i> | KUMCC 17-0163 * | On dead wood | China | S.K. Huang | MG746632 | – | – | MG746633 | MG746634 |
| <i>D. taoicola</i> | MFLUCC 16-0117 * | <i>Prunus persica</i> | China | X.H. Li | KU557567 | – | – | KU557635 | KU557591 |
| | MFLUCC 16-0118 | <i>Prunus persica</i> | China | X.H. Li | KU557568 | – | – | KU557636 | KU557591 |
| <i>D. tectonae</i> | MFLUCC 12-0777 * | <i>Tectonia grandis</i> | China | – | KU712430 | KU749345 | – | KU749359 | KU743977 |
| <i>D. tectonendophytica</i> | MFLUCC 13-0471 * | <i>Tectonia grandis</i> | Thailand | – | KU712439 | KU749354 | KX999266 | KU749367 | KU743986 |
| <i>D. tectonigena</i> | MFLUCC 12-0767 = ICMP 21169 * | <i>Tectonia grandis</i> | Thailand | M. Doilom | KU712429 | KU749358 | – | KU749371 | KU743976 |
| <i>D. thunbergiicola</i> | MFLUCC 12-0033 * | <i>Thunbergia laurifolia</i> | Thailand | D. Udayanga | KP715097 | – | – | KP715098 | – |
| <i>D. tibetensis</i> | CFCC 51999 * | <i>Juglandis regia</i> | China | X.L. Fan | MF279843 | MF279888 | MF279828 | MF279858 | MF279873 |
| <i>D. tulliensis</i> | BRIP 62248a * | <i>Theobroma cacao</i> fruit | Australia | – | KR936130 | – | – | KR936133 | KR936132 |
| <i>D. ueckeriae</i> | FAU 656 * | <i>Cucumis melo</i> | USA | – | KJ590726 | KJ612122 | KJ659215 | KJ590747 | KJ610881 |
| <i>D. ukurunduensis</i> | CFCC 52592 * | <i>Acer ukurunduense</i> | China | Q. Yang | MH121527 | MH121445 | MH121485 | MH121569 | – |
| <i>D. undulata</i> | CGMCC 3.18293 = LC6624 * | Leaf of unknown host | China-Laos border | F. Liu | KX986798 | – | KX999269 | KX999190 | KX999230 |
| <i>D. unshuiensis</i> | CGMCC 3.17569 = ZJUD52 * | <i>Citrus unshiu</i> | China | G.Q. Chen | KJ490587 | – | KJ490529 | KJ490466 | KJ490408 |
| | CGMCC 3.17568 = ZJUD51 | <i>Citrus japonica</i> | China | F. Huang | KJ490586 | – | KJ490528 | KJ490465 | KJ490407 |
| <i>D. vaccinii</i> | CBS 160.32 = IFO 32646 * | <i>Oxycoccus macrocarpos</i> | USA | C.L. Shear | KC343228 | KC343470 | KC343712 | KC343954 | KC344196 |
| <i>D. velutina</i> | CGMCC 3.18286 = LC 4421 * | <i>Neolitsea</i> sp. | China | Y.H. Gao | KX986790 | – | – | KX999182 | KX999223 |
| | PSCG 134 | <i>Pyrus pyrifolia</i> | China | – | MK626918 | MK691173 | – | MK654853 | MK691243 |
| <i>D. virgiliae</i> | GMW40748 | <i>Virgilia oroboides</i> | South Africa | – | KP247566 | – | – | KP247575 | – |
| <i>D. xishuangbanica</i> | CGMCC 3.18282 = LC6707 * | <i>Camellia sinensis</i> | China | F. Liu | KX986783 | – | KX999255 | KX999175 | KX999216 |
| <i>D. yunnanensis</i> | CGMCC 3.18289 = LC6168 * | <i>Coffea</i> sp. | China | W.J. Duan | KX986796 | KX999290 | KX999267 | KX999188 | KX999228 |
| <i>D. zaofenghuang</i> | CGMCC 3.20271 = TZFH1 * | <i>Prunus persica</i> | China | Y.S. Guo | MW477883 | MW480867 | MW480863 | MW480871 | MW480875 |
| | TZFH3 | <i>Prunus persica</i> | China | Y.S. Guo | MW477884 | MW480868 | MW480864 | MW480872 | MW480876 |
| <i>Diaporthea corylina</i> | CBS 121124 * | <i>Corylus</i> sp. | China | L.N. Vassiljeva | KC343004 | KC343246 | KC343488 | KC343730 | KC343972 |

^a AR, DP, FAU: Culture collection of Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, Maryland, USA; ATCC: American Type Culture Collection, Virginia, USA; BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CAA: Personal culture collection Artur Alves, Universidade de Aveiro, Portugal; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, South Africa; CPC: Working collection of P.W. Crous, housed at CBS; DNP: Personal collection of Dhanushka Udayanga; ICMP: International Collection of Micro-organisms from Plants, Auckland, New Zealand; LC: Personal collection number of Lei Cai, State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences; JZB: JZB culture collection in Institute of Plant and Environment Protection of Beijing Academy of Agriculture and Forestry Sciences, Beijing, China; MAFF: MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MFLU: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; PSCG: Personal culture collection, Yashuang Guo, State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Hubei, China; SAUCC: Shandong Agricultural University, Shandong, China; ZJUD: Personal culture collection of Feng Huang, Institute of Biotechnology, Zhejiang University, Zhejiang, China.

^b ITS: internal transcribed spacer region and intervening 5.8S rDNA gene; cal, calmodulin; his3, histone h3; tef1, translation elongation factor 1-alpha; tub2, beta-tubulin.
* Isolates represent ex-type.

RESULTS

Sampling and isolation

In the surveyed citrus orchards, we collected twigs and branches that withered from top to bottom, sometimes accompanied by a small amount of gum exudation, and in severe cases, the whole dead branch (Fig. 1a, b). The affected branch and trunk tissues appeared cracked, with dark discoloration, exuding light brown gum, and as the disease progressed, lesions gradually dried up and sank (Fig. 1c–f), and a large number of protruding small granules (conidiomata) formed on the dead branch in the late stage of disease development (Fig. 1h). The pathogenic fungus colonized the bark of sunburned or frostbitten trucks or branches, then invaded the xylem, and gradually extended upwards and downwards (Fig. 1g). Light brown sunken dots, surrounded by yellow halos, appeared on the surface of newly generated leaves and shoots in the early stage of being affected (Fig. 1i), and later the yellow halos

disappeared and turned into black raised dots (Fig. 1j, k). Scattered black or reddish brown protruding small dots appeared when symptoms on the fruit surface were mild (Fig. 1l), and the small dots merged to form flaky lesions when the infection was severe (Fig. 1m).

A total of 1 103 isolates were recovered from leaves, shoots and fruits with melanose symptoms. These *Diaporthe* isolates were initially identified using *D. citri*-specific primers (Zeng et al. 2022) and 894 isolates identified as *D. citri*. The remaining 209 isolates initially identified as non-*D. citri* and 184 of these *Diaporthe* isolates from diseased twigs, branches and trunks were included for further study. Among them, 14 isolates were collected from Chongqing, 19 from Fujian, 18 from Guangdong, 12 from Guizhou, 139 from Hunan, 26 from Jiangxi, 21 isolates from Shanghai, two from Sichuan, nine from Yunnan, and 133 from Zhejiang. In terms of *Diaporthe* isolate distribution among host *Citrus* tree species, 15 were recovered from *C. limon*, 71 from *C. maxima*, 81 from *C. reticulata*, 73 from *C. sinensis*, 118



Fig. 1 Disease symptoms on *Citrus* caused by *Diaporthe*. a. Twig blight; b. branch dieback; c–d. gummosis on branch and trunk; e–f. gummosis and decay on trunk, peeled off the bark and a clear discoloration of the xylem could be observed; g. gummosis and decay on trunk; h. conidiomata formed on dead branch (white arrow indicates a conidioma); i. melonose initially developed in leaf with small brown spots surrounded by yellow halo; j–k. dark brown to black and raised spots formed on leaves and shoot caused by melonose; l. melonose on citrus fruit; m. the small dots on the fruit are dense in patches when disease is severe.

from *C. unshiu*, three from *F. margarita*, four from *P. trifoliata*, and 28 from citrus hybrids. As for symptoms, 81 isolates were obtained from twig blight and gummosis; 75 associated with branch dieback, canker and gummosis; 26 from trunk canker, decay and gummosis; two from asymptomatic twig; 139 associated with leaf melanose; nine from shoot melanose, and 61 from fruit melanose. Most of these symptoms were observed on most citrus varieties (Table 4, S1).

Phylogenetic analyses

The ITS sequences were amplified for all 393 isolates obtained in this study. Based on geography and host tree affiliations, representative isolates were selected from isolates with identical ITS and *tub2* sequences, and then 131 isolates were selected in the same manner for their *cal*, *his3* and *tef1* sequences (Table 2). The partition homogeneity test for both four- and five-loci combined alignments generated a low *p*-value ($p = 0.01$), indicating that these genes were unsuitable for combination. Although the *p*-values were low, sequences of the four and five regions were combined for presentation purposes. Phylogenetic analyses were performed based on the concatenation of multigene sequence data for the 131 isolates obtained in this study and 148 related strains downloaded from NCBI as reference strains (Table 3).

In the *D. eres* species complex, phylogenetic analyses based on the four genes (*cal*, *his3*, *tef1*, *tub2*) consisted of 59 sequences, including the outgroup sequences of *D. citri* (CBS 135422), with a total of 1481 characters including gaps (*cal*:

1–343, *his3*: 344–783, *tef1*: 784–1123, *tub2*: 1124–1481). For the BI analyses, MrModeltest recommended model K80 for *cal*, model HKY+I for *his3* and model HKY+G for *tef1* and *tub2*. In the phylogenetic construction based on four loci, 23 isolates clustered in the *D. eres* species complex, of which 19 isolates resided in one of the clades within the *D. eres* species complex and four isolates grouped with *D. penetriseum* (Fig. 2).

For the remaining *Diaporthe* spp., phylogenetic analyses based on the five genes (ITS, *cal*, *his3*, *tef1*, *tub2*) consisted of 256 sequences, including the outgroup sequences of *Diaporthe corylina* (CBS 121124), with a total of 2352 characters including gaps (ITS: 1–517, *cal*: 518–914, *his3*: 915–1420, *tef1*: 1421–1913, *tub2*: 1914–2352). For the BI analyses, MrModeltest recommended models GTR+I+G for ITS, *his3* and *tef1*, and HKY+I+G for *cal* and *tub2*. In the phylogenetic analyses based on five loci, 92 isolates were clustered with 30 species respectively, including *D. apiculata* (4 isolates), *D. biconispora* (1), *D. biguttulata* (3), *D. caryae* (2), *D. citri* (11), *D. citriasiana* (5), *D. compacta* (6), *D. endophytica* (3), *D. discoidispora* (3), *D. fusicola* (1), *D. fulvicolor* (2), *D. guangxiensis* (1), *D. hongkongensis* (7), *D. hubeiensis* (2), *D. limonicola* (1), *D. litchii* (3), *D. novem* (1), *D. passifloricola* (1), *D. pescicola* (1), *D. pometaiae* (4), *D. sackstonii* (4), *D. sennicola* (1), *D. sojae* (5), *D. spinosa* (1), *D. subclavata* (5), *D. tectonae* (1), *D. tibetensis* (1), *D. unshiuensis* (4), *D. velutina* (7) and *D. xishuangbanica* (1). In addition, 16 isolates formed four lineages distinct from the other species (Fig. 3). These four distinct lineages were therefore considered novel species of *Diaporthe*, which are described below.

Table 4 Number of isolates collected for each *Diaporthe* species identified and province investigated.

| | Chongqing | Fujian | Guangdong | Guizhou | Hunan | Jiangxi | Shanghai | Sichuan | Yunnan | Zhejiang | Total |
|--------------------------|-----------|--------|-----------|---------|-------|---------|----------|---------|--------|----------|-------|
| <i>D. apiculata</i> | – | – | – | 2 | 1 | 1 | – | – | – | – | 4 |
| <i>D. biguttulata</i> | – | – | – | – | 6 | – | – | – | – | – | 6 |
| <i>D. biconispora</i> | – | – | – | – | 1 | 1 | – | – | – | – | 2 |
| <i>D. caryae</i> | – | – | – | – | 3 | – | – | – | – | – | 3 |
| <i>D. citri</i> | 1 | 3 | 8 | 5 | 48 | 10 | 19 | – | 1 | 97 | 192 |
| <i>D. citriasiana</i> | – | – | – | – | 4 | – | – | – | – | 19 | 23 |
| <i>D. compacta</i> | – | – | – | – | 6 | – | – | – | – | – | 6 |
| <i>D. discoidispora</i> | – | – | – | – | 7 | – | – | – | – | 1 | 8 |
| <i>D. endophytica</i> | 2 | 1 | – | – | – | – | – | – | – | – | 3 |
| <i>D. eres</i> | 2 | – | – | 1 | 16 | 7 | 1 | – | 1 | 8 | 36 |
| <i>D. fulvicolor</i> | 4 | – | – | – | – | – | – | – | – | – | 4 |
| <i>D. fusicola</i> | – | – | – | – | 1 | – | – | – | – | – | 1 |
| <i>D. gammata</i> | 2 | – | – | – | 1 | – | – | – | – | – | 3 |
| <i>D. guangxiensis</i> | – | – | – | – | – | 1 | – | – | – | – | 1 |
| <i>D. hongkongensis</i> | – | 1 | 1 | 1 | 11 | 1 | – | – | – | – | 15 |
| <i>D. hubeiensis</i> | – | – | – | – | 2 | – | – | – | – | – | 2 |
| <i>D. jishouensis</i> | – | 1 | – | 1 | 8 | 1 | – | – | – | – | 11 |
| <i>D. limonicola</i> | – | – | – | – | – | – | 1 | – | – | – | 1 |
| <i>D. litchii</i> | – | – | 6 | – | – | – | – | – | – | – | 6 |
| <i>D. novem</i> | – | – | – | – | – | – | – | – | 1 | – | 1 |
| <i>D. passifloricola</i> | – | 1 | – | – | – | – | – | – | – | – | 1 |
| <i>D. penetriseum</i> | – | – | – | 1 | 2 | – | – | – | 2 | – | 5 |
| <i>D. pescicola</i> | – | 1 | – | – | – | – | – | – | – | – | 1 |
| <i>D. pometaiae</i> | – | 8 | 1 | – | – | – | – | – | – | – | 9 |
| <i>D. ruiliensis</i> | – | – | – | – | – | – | – | – | 1 | – | 1 |
| <i>D. sackstonii</i> | 2 | – | – | – | 3 | – | – | 2 | 1 | – | 8 |
| <i>D. sennicola</i> | – | – | 1 | – | – | – | – | – | – | – | 1 |
| <i>D. sexualispora</i> | – | – | – | – | 1 | – | – | – | – | – | 1 |
| <i>D. sojae</i> | 1 | – | – | 1 | 8 | 2 | – | – | – | 2 | 14 |
| <i>D. spinosa</i> | – | – | – | – | – | – | – | – | – | 1 | 1 |
| <i>D. subclavata</i> | – | 1 | – | – | 4 | – | – | – | – | – | 5 |
| <i>D. tectonae</i> | – | – | – | – | 1 | – | – | – | – | – | 1 |
| <i>D. tibetensis</i> | – | – | – | – | 1 | – | – | – | – | – | 1 |
| <i>D. unshiuensis</i> | – | 2 | – | – | 2 | 1 | – | – | 2 | 1 | 8 |
| <i>D. velutina</i> | – | – | – | – | 2 | 1 | – | – | – | 4 | 7 |
| <i>D. xishuangbanica</i> | – | – | 1 | – | – | – | – | – | – | – | 1 |
| Total | 14 | 19 | 18 | 12 | 139 | 26 | 21 | 2 | 9 | 133 | 393 |



Fig. 2 Phylogenetic tree generated by Bayesian inference analyses based on multiple gene loci (*cal*, *his3*, *tef1*, *tub2*) for species in the *D. eres* species complex. Bayesian posterior probability (PP ≥ 0.9) and IQtree bootstrap support values (ML $\geq 50\%$) presented above branches as follows: PP/ML. PP < 0.9 and ML $< 50\%$ of branches marked with *, and absent marked with -. Ex-type strains in bold. The tree is rooted to *Diaporthe citri* (CBS 135422). The clades marked with colour blocks contain isolates of *Diaporthe* obtained in this study.

Taxonomy

Diaporthe gammata X.E. Xiao, Crous & H.Y. Li, *sp. nov.* — MycoBank MB 847573; Fig. 4

Etymology. Named for the presence of gamma conidia.

Typus. CHINA, Chongqing Municipality, Fengjie County, from twig dieback of *Citrus reticulata*, 2 Apr. 2019, T.J. Huang (holotype ZJUE H-0318, culture ex-type CGMCC3.24384 = ZJUE 0318).

Sexual morph not observed. *Conidiomata* pycnidial, produced on PNA, dark brown to black, globose or irregular, superficial or semi-immersed, solitary or aggregated, 300–820 μm diam. *Conidiophores* hyaline, smooth, 1–2-septate, unbranched, cylindrical, 9–27 \times 1.5–3 μm . *Conidiogenous cells* phialidic, hyaline, cylindrical, tapered towards the apex, 5–11 \times 1–4 μm .

Alpha conidia aseptate, ellipsoid to cylindrical, obtusely rounded at both ends, hyaline, biguttulate, 5.5–10 \times 1.5–3 μm , av. \pm SD = 7.7 \pm 1.1 \times 2.5 \pm 0.3 μm , L/W ratio = 3.1 (n = 50). *Beta conidia* hyaline, aseptate, filiform, straight or curved, tapering towards both ends, 29–48.5 \times 1–2 μm , av. \pm SD = 38.2 \pm 1.6 \times 1.6 \pm 0.2 μm , L/W ratio = 23.9 (n = 50). *Gamma conidia* not observed on PNA but on PDA, hyaline, aseptate, fusoid to obclavate, tapering towards both ends, 16–31.5 \times 1.5–4 μm , av. \pm SD = 24.3 \pm 4 \times 2.8 \pm 0.4 μm , L/W ratio = 8.7 (n = 50).

Culture characteristics — Colonies on PDA with fluffy aerial mycelium, growing in a concentric ring pattern, initially white, turning grey olivaceous at the centre, becoming dark brick on the surface and sepia to black in reverse, conidiomata distributed in a concentric ring pattern. On MEA with flattened and dense mycelium, developing in a whorl pattern, initially white,

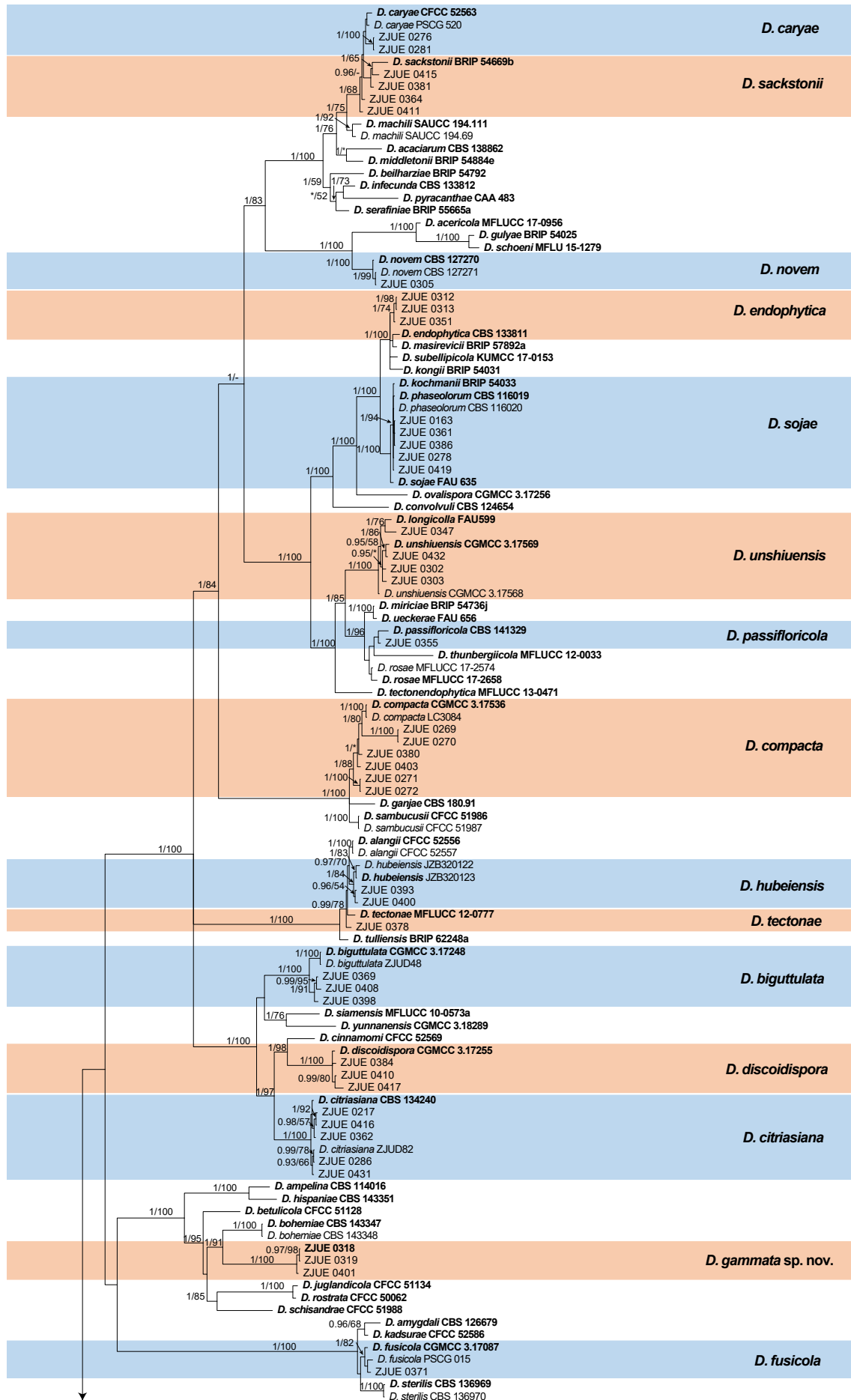


Fig. 3 Phylogenetic tree generated by Bayesian inference analyses based on multiple gene loci (ITS, *cal*, *his3*, *tef1*, *tub2*) for species in *Diaporthe* spp. Bayesian posterior probability (PP ≥ 0.9) and IQtree bootstrap support values (ML ≥ 50 %) presented above branches as follows: PP/ML. PP < 0.9 and ML < 50 % of branches marked with *, and absent marked with -. Ex-type strains in bold. The tree is rooted to *Diaporthella corylina* (CBS 121124). The clades marked with colour blocks contain the isolates of *Diaporthe* obtained in this study.

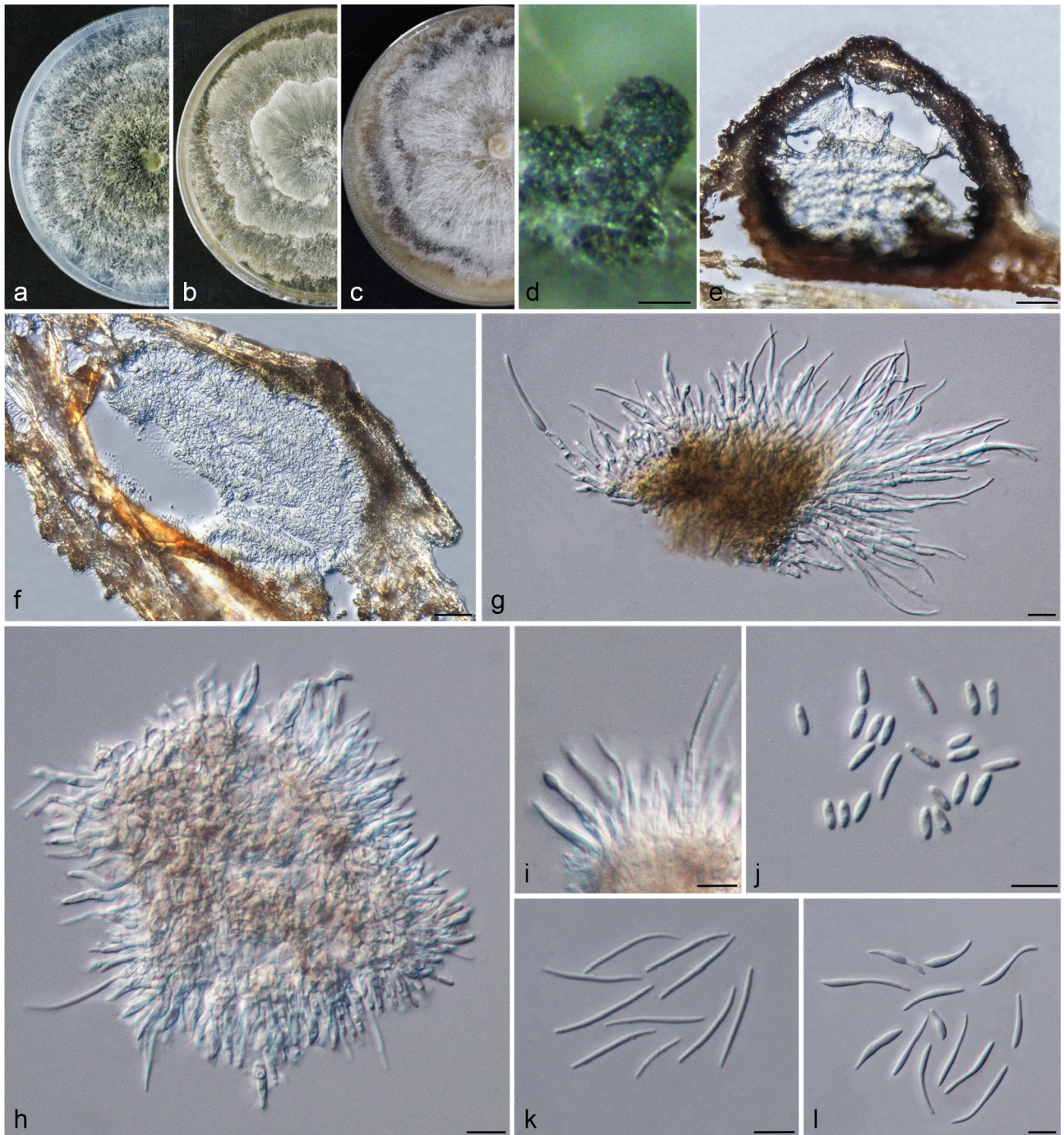


Fig. 4 *Diaporthe gammata*. a–c. Colonies on PDA, MEA and OA, respectively; d. conidiomata formed on PNA; e, f. section view of conidiomata; g–i. conidiophores; j. alpha conidia; k. beta conidia; l. gamma conidia. — Scale bars: d = 200 μ m; e, f = 50 μ m; g–l = 10 μ m.

being hazel on the surface, olivaceous in the reverse, and brown vinaceous in the whorl, conidiomata distributed in the whorl. On OA with fluffy aerial mycelium, growing in a petaloid pattern, initially white, turning ochreous at the centre, brown vinaceous pigment irregularly distributed in the petaloid pattern, reverse with umber at the centre and dark mouse grey at the margin, conidiomata distributed in the petaloid pattern irregularly. Colony diam 50–51 mm, 39–42 mm and 50–55 mm on PDA, MEA and OA, respectively, in 5 d at 25 °C.

Additional materials examined. CHINA, Chongqing Municipality, Fengjie County, from twig dieback of *C. reticulata*, 2 Apr. 2019, T.J. Huang (culture ZJUE 0319); Hunan Province, Luxi County, from leaf with melanose of *C. reticulata*, 22 Aug. 2019, S.S. Tan & D.H. Li (culture ZJUE 0401).

Notes — Three isolates represent the *D. gammata* cluster in a clade distinct from other species of *Diaporthe* known from

DNA sequence data. Phylogenetically, *D. gammata* is most closely related to *D. bohemiae* (Fig. 3, S2a–e), but can be distinguished from *D. bohemiae* based on morphology in the production of beta and gamma conidia, which are not observed in *D. bohemiae* (Guarnaccia et al. 2018) (Table 5). Moreover, *D. gammata* differs from *D. bohemiae* in sequence similarity on ITS (98.6 %), *cal* (90.4 %), *his3* (91.3 %), *tef1* (83.7 %) and *tub2* (96.9 %).

Diaporthe jishouensis X.E. Xiao, Crous & H.Y. Li, *sp. nov.* — MycoBank MB 847574; Fig. 5

Etymology. Referring to Jishou City where the fungus was isolated.

Typus. CHINA, Hunan Province, Jishou City, from twig dieback of *Citrus unshiu*, 31 Mar. 2019, H.Y. Li (holotype ZJUE H-0265, culture ex-type CG-MCC3.24382 = ZJUE 0265).



Fig. 5 *Diaporthe jishouensis*. a–c. Colonies on PDA, MEA and OA, respectively; d. pale yellow conidial mass oozing from conidiomata formed on PNA; e, f. section view of conidiomata; g–i. conidiophores; j. alpha and beta conidia. — Scale bars: d = 400 μ m; e, f = 50 μ m; g–j = 10 μ m.

Sexual morph not observed. **Conidiomata** pycnidial, produced on PNA, dark brown to black, globose or irregular, superficial or semi-immersed, solitary or aggregated, 175–750 μ m diam, often releasing pale yellow conidial drops from the ostioles. **Conidiophores** hyaline, smooth, 1-septate, unbranched, cylindrical, 9.5–18 \times 1.5–3 μ m. **Conidiogenous cells** phialidic, hyaline, cylindrical, tapered towards the apex, 5–13.5 \times 1–3 μ m. **Alpha conidia** aseptate, ellipsoidal to cylindrical, obtusely rounded at both ends or acutely round at one end, hyaline, bi- or multi-guttulate, 5.5–9 \times 1.5–3.5 μ m, av. \pm SD = 7.2 \pm 0.6 \times 2.7 \pm 0.3 μ m, L/W ratio = 2.7 (n = 50). **Beta conidia** hyaline, aseptate, filiform, straight or curved, tapering towards both ends, 30.5–54.5 \times 1–2 μ m, av. \pm SD = 44.9 \pm 5 \times 1.6 \pm 0.2 μ m, L/W ratio = 28.1 (n = 50). **Gamma conidia** not observed.

Culture characteristics — Colonies on PDA and MEA have fluffy aerial mycelium, initially white, becoming dark yellow with age, conidiomata releasing cream white to pale yellow conidial drops, reverse with sepia pigmentation in a concentric ring pattern. On OA with few aerial mycelium, initially white, producing dark brick pigment soon, becoming dense, felted, pale greenish olivaceous at the centre and vinaceous buff at the margin, conidiomata releasing cream white to pale yellow conidial drops, reverse with hazel. Colony diam 79–82 mm, 25–39 mm and 42–47 mm on PDA, MEA and OA, respectively, in 5 d at 25 $^{\circ}$ C.

Additional materials examined. CHINA, Hunan Province, Jishou City, from twig dieback of *C. reticulata*, 31 Mar. 2019, H.Y. Li (culture ZJUE 0291); Fujian Province, Zhangzhou City, from leaf with melanose of *C. maxima*, 2 July 2019, T. Xiong (culture ZJUE 0353); Guizhou Province, Congjiang County, from leaf with melanose of *C. reticulata*, 8 June 2019, J.R. Long & G.F. Liang (culture ZJUE 0356); Hunan Province, Luxi County, from leaf with melanose of *C. sinensis*, 24 Aug. 2019, S.S. Tan & D.H. Li (culture ZJUE 0372, ZJUE 0374, ZJUE 0376, ZJUE 0382); Hunan Province, Shaoyang City, from leaf with melanose of *C. reticulata*, 21 Aug. 2019, Y.T. Zeng (culture ZJUE 0421); Jiangxi Province, Ganzhou City, from leaf with melanose of *C. sinensis*, 25 May 2019, X. Yan (culture ZJUE 0423).

Notes — Phylogenetically, *D. jishouensis* is closely related to *D. incompleta*, *D. ruiliensis* and *D. shaanxiensis* (Fig. 3, S2a–e), but can be distinguished from these species based on morphology. *Diaporthe jishouensis* produces alpha conidia, which are not known in *D. incompleta* (Gao et al. 2017) and *D. shaanxiensis* (Yang et al. 2020), and beta conidia of *D. jishouensis* are longer than in *D. incompleta* (30.5–54.5 vs 19–44 μ m) (Gao et al. 2017) and *D. ruiliensis* (30.5–54.5 vs 29.5–47 μ m) (this study) (Table 5). Moreover, *D. jishouensis* has the following sequence similarities with the sequences of the ex-types of *D. incompleta*, *D. ruiliensis* and *D. shaanxiensis*. On ITS: 98.2 %, 96.9 % and 98.4 %, respectively. On *cal*: 95.5 %, 95.3 % and 92.8 %, respectively. On *his3*: 87.5 %, 94.4 % and 94.9 %, respectively. On *tef1*: 94 %, 94.1 % and 93.9 %, respectively. On *tub2*: 87.4 %, 97.2 % and no *tub2* for *D. shaanxiensis*, respectively.

Table 5 Conidial measurements of novel *Diaporthe* species and their phylogenetically closely related species in this study.

| Species | Alpha conidia (μm) | Beta conidia (μm) | Gamma conidia (μm) | Reference |
|-------------------------------|--|--|--|--------------------------|
| <i>D. bohemiae</i> | 7.5–8.5 \times 1.5–3 | – | – | Guarnaccia et al. (2018) |
| <i>D. diospyricola</i> | (5.5–)6–7(–7.5) \times (2–)2.5(–3) | (18–)25–27(–30) \times 1.5(–2) | – | Crous et al. (2013) |
| <i>D. gammata</i> | 5.5–10 \times 1.5–3 | 29–48.5 \times 1–2 | 16–31.5 \times 1.5–4 | This study |
| <i>D. incompleta</i> | – | 19–44 \times 0.5–1.5 | – | Gao et al. (2017) |
| <i>D. jishouensis</i> | 5.5–9 \times 1.5–3.5 | 30.5–54.5 \times 1–2 | – | This study |
| <i>D. oncostoma</i> | (7.5–)9–11(–12) \times (2–)3(–4) | – | (11–)12–16 \times 3(–3.5) | Crous et al. (2013) |
| <i>D. ruiiisensis</i> | 5.5–9 \times 2–3 | 29.5–47 \times 1–2 | – | This study |
| <i>D. saccharata</i> | (11–)16–20(–24) \times (2.5–)3(–4) | (15–)18–20(–27) \times (1–)1.5(–2) | – | Mostert et al. (2001b) |
| <i>D. sexualispora</i> | 8–12 \times 3–4.5 | 15–29 \times 1.5–2.5 | – | This study |
| <i>D. shaanxiensis</i> | – | (35.5–)37–47.5(–50) \times 1 | – | Yang et al. (2020) |

* Isolates and measurements in **bold** were examined in this study.**Fig. 6** *Diaporthe ruiiisensis*. a–c. Colonies on PDA, MEA and OA, respectively; d. yellow conidial mass oozing from conidiomata formed on PNA; e, f. section view of conidiomata; g–i. conidiophores and beta conidia developing on conidiophores; j. alpha conidia; k. beta conidia. — Scale bars: d = 400 μm ; e, f = 50 μm ; g–k = 10 μm .

Diaporthe ruii X.E. Xiao, Crous & H.Y. Li, *sp. nov.* — MycoBank MB 847575; Fig. 6

Etymology. Referring to the Ruii City where the fungus was isolated.

Typus. CHINA, Yunnan Province, Ruii City, from twig dieback of *Citrus limon*, 2 Apr. 2018, H.Y. Li (holotype ZJUE H-0307, culture ex-type CG-MCC3.24383 = ZJUE 0307).

Sexual morph not observed. *Conidiomata* pycnidial, produced on PNA, dark brown to black, globose or irregular, superficial or semi-immersed, solitary or aggregated, 145–560 µm diam, often releasing in pale yellow to saffron yellow conidial drops. *Conidiophores* hyaline, smooth, 1-septate, unbranched, cylindrical, 8–18.5 × 1.5–3.5 µm. *Conidiogenous cells* phialidic, hyaline, cylindrical, tapered towards the apex, 5–14.5 × 1.5–4 µm. *Alpha conidia* aseptate, ellipsoidal to cylindrical, obtusely rounded at both ends, hyaline, bi- or multi-guttulate, 5.5–9 × 2–3 µm, av. ± SD = 7.4 ± 0.6 × 2.5 ± 0.2 µm, L/W ratio = 3 (n = 50). *Beta conidia* hyaline, aseptate, filiform, straight or curved, tapering towards both ends, 29.5–47 × 1–2 µm, av. ± SD = 37.3 ± 3.6 × 1.4 ± 0.1 µm, L/W ratio = 26.6 (n = 50). *Gamma conidia* not observed.

Culture characteristics — Colony on PDA with white, felted and dense aerial mycelium developing in a feathery pattern, being fluffy, greyish sepia on the surface and sepia to fuscous black in the reverse. On MEA with white felted and dense mycelium developing in a feathery pattern, turning smoke grey, reverse olivaceous. On OA with white dense and felted aerial mycelium on the surface, turning vinaceous buff at the centre and honey at the margin in the reverse. Colony diam 40–41 mm, 24–32 mm and 42–47 mm on PDA, MEA and OA, respectively, in 5 d at 25 °C.

Notes — Phylogenetically, *D. ruii* forms an independent lineage, closely related to *D. incompleta*, *D. jishouensis* and *D. shaanxiensis* (Fig. 3, S2a–e), but can be differentiated from these species based on morphology. Beta conidia of *D. ruii* are longer than *D. incompleta* (29.5–47 vs 19–44 µm) (Gao et al. 2017) but shorter than *D. jishouensis* (29.5–47 vs 30.5–54.5 µm) and *D. shaanxiensis* (29.5–47 vs 35.5–50 µm) (Yang et al. 2020) (Table 5). Moreover, *D. ruii* has the following sequence similarities with the sequences of the ex-types of *D. incompleta*, *D. jishouensis* and *D. shaanxiensis*. On ITS: 95.2 %, 96.9 % and 92.5 %, respectively. On *cal*: 99.8 %, 95.3 % and 93.3 %, respectively. On *his3*: 85.1 %, 94.4 % and 94.1 %, respectively. On *tef1*: 100 %, 94.1 % and 92 %, respectively. On *tub2*: 86.2 %, 97.2 % and no *tub2* for *D. shaanxiensis*.

Diaporthe sexualispora X.E. Xiao, Crous & H.Y. Li, *sp. nov.* — MycoBank MB 847578; Fig. 7

Etymology. Named for the presence of its sexual morph.

Typus. CHINA, Hunan Province, Shaoyang City, from leaf with melanose of *Citrus unshiu*, 20 Aug. 2019, Y.T. Zeng (holotype ZJUE H-0418, culture ex-type CGMCC3.24385 = ZJUE 0418).

Ascomata produced on PNA, dark brown to black, ostiolate, 250–750 µm diam, deeply immersed, densely clustered in groups, multiple tapered, spiny perithecial necks producing through substrate, 890–1710 µm long. *Asci* hyaline, unitunicate, fasciculate, sessile, elongate to clavate, 42.5–57 × 7.5–10 µm. *Ascospores* hyaline, two-celled, ellipsoid to fusoid, often 4-guttulate, with larger guttules at centre and smaller ones at the ends, 10–16.5 × 10–4 µm. *Conidiomata* pycnidial, produced on PNA and PDA, dark brown to black, globose or irregular, superficial or semi-immersed, solitary or aggregated, 290–400 µm diam. *Conidiophores* hyaline, smooth, 1-septate, unbranched, cylindrical, 9–19.5 × 1.5–4 µm. *Conidiogenous*

cells phialidic, hyaline, cylindrical, tapered towards the apex, 7.5–12.5 × 2–4 µm. *Alpha conidia* aseptate, fusoid to ovoid, acutely rounded at both ends, hyaline, biguttulate, 8–12 × 3–4.5 µm, av. ± SD = 10.2 ± 0.9 × 3.8 ± 0.3 µm, L/W ratio = 2.7 (n = 50). *Beta conidia* hyaline, aseptate, filiform, straight or curved, tapering towards one end, 15–29 × 1.5–2.5 µm, av. ± SD = 20.8 ± 2.9 × 2.1 ± 0.2 µm, L/W ratio = 9.9 (n = 50). *Gamma conidia* not observed.

Culture characteristics — Colony on PDA with felted and dense aerial mycelium, developing in a feathery pattern, initially white, reverse with honey pigmentation with age, conidiomata releasing straw-coloured conidial drops at maturity. On MEA white, felted and with dense mycelium, growing in a feathery pattern, reverse honey with buff pigments, conidiomata excreting straw conidial droplets at maturity. On OA with felted and dense mycelium, growing in a radial pattern, initially white, turning white to honey, becoming buff at the centre on the surface and buff in the reverse, conidiomata releasing cream white to yellow conidial drops at maturity. Colony diam 30–34 mm, 19–20 mm and 39–41 mm on PDA, MEA and OA, respectively, in 5 d at 25 °C.

Notes — Phylogenetically, *D. sexualispora* forms an independent lineage closely related to *D. diospyricola*, *D. oncostoma* and *D. saccharata* (Fig. 3, S2a–e), but can be distinguished from these species based on morphology. Alpha conidia of *D. sexualispora* are shorter than *D. saccharata* (8–16 vs 11–24 µm) (Mostert et al. 2001b) but larger than *D. diospyricola* (8–16 × 3–4.5 vs 5.5–7.5 × 2–3 µm) (Crous et al. 2013) and *D. oncostoma* (8–12 × 3–4.5 vs 7.5–11 × 2–4 µm) (Crous et al. 2013). Besides, *D. sexualispora* differs from *D. oncostoma* in the production of beta conidia not observed in *D. oncostoma* (Crous et al. 2013) (Table 5). Moreover, *D. sexualispora* has the following sequence similarities with the sequences of the ex-types of *D. diospyricola*, *D. oncostoma* and *D. saccharata*. On ITS: 95.7 %, 96.2 % and 95.5 %, respectively. On *cal*: no *cal* for *D. diospyricola*, 92.3 % and 88.9 %, respectively. On *his3*: no *his3* for *D. diospyricola*, 90.2 % and 92.7 %, respectively. On *tef1*: no *tef1* for *D. diospyricola*, 87.5 % and 82.1 %, respectively. On *tub2*: no *tub2* for *D. diospyricola*, 92.8 % and 92.5 %, respectively.

Species associated with disease symptoms

In total, 36 species of *Diaporthe* were identified from citrus tissues in this study. Of these species, four were obtained from branch diseases with symptoms of dieback, decay, canker and gummosis, 19 recovered from melanose symptoms, and 13 from both types of symptoms (Table S1). *Diaporthe citri* was the dominant species for both symptom types, accounting for 71.7 % of the isolates obtained from branch diseases and 86.5 % from melanose symptoms.

Pathogenicity

For pathogenicity tests via *C. paradisi* shoot inoculations, all the tested *Diaporthe* isolates could induce discoloured and necrotic lesions 12 d post inoculation. The lesion lengths varied significantly among the tested isolates. Overall, both isolates of *D. citri* produced the longest lesions, with an average lesion length of more than 9 cm. Isolate ZJUE 0378 of *D. tectonae*, ZJUE 0393 and ZJUE 0400 of *D. hubeiensis*, ZJUE 0418 of *D. sexualispora*, ZJUE 0217 and ZJUE 0416 of *D. citriasiana* produced moderate-length lesions (4 cm < mean lesion length < 9 cm). In contrast, the remaining *Diaporthe* isolates induced very limited lesions (mean lesion length < 4 cm), especially isolates of *D. apiculata*, *D. bioconispora* and 13 other species caused only slight discolouration around the inoculation points. *Diaporthe* isolates produced shorter lesions on *C. reticulata* cv.

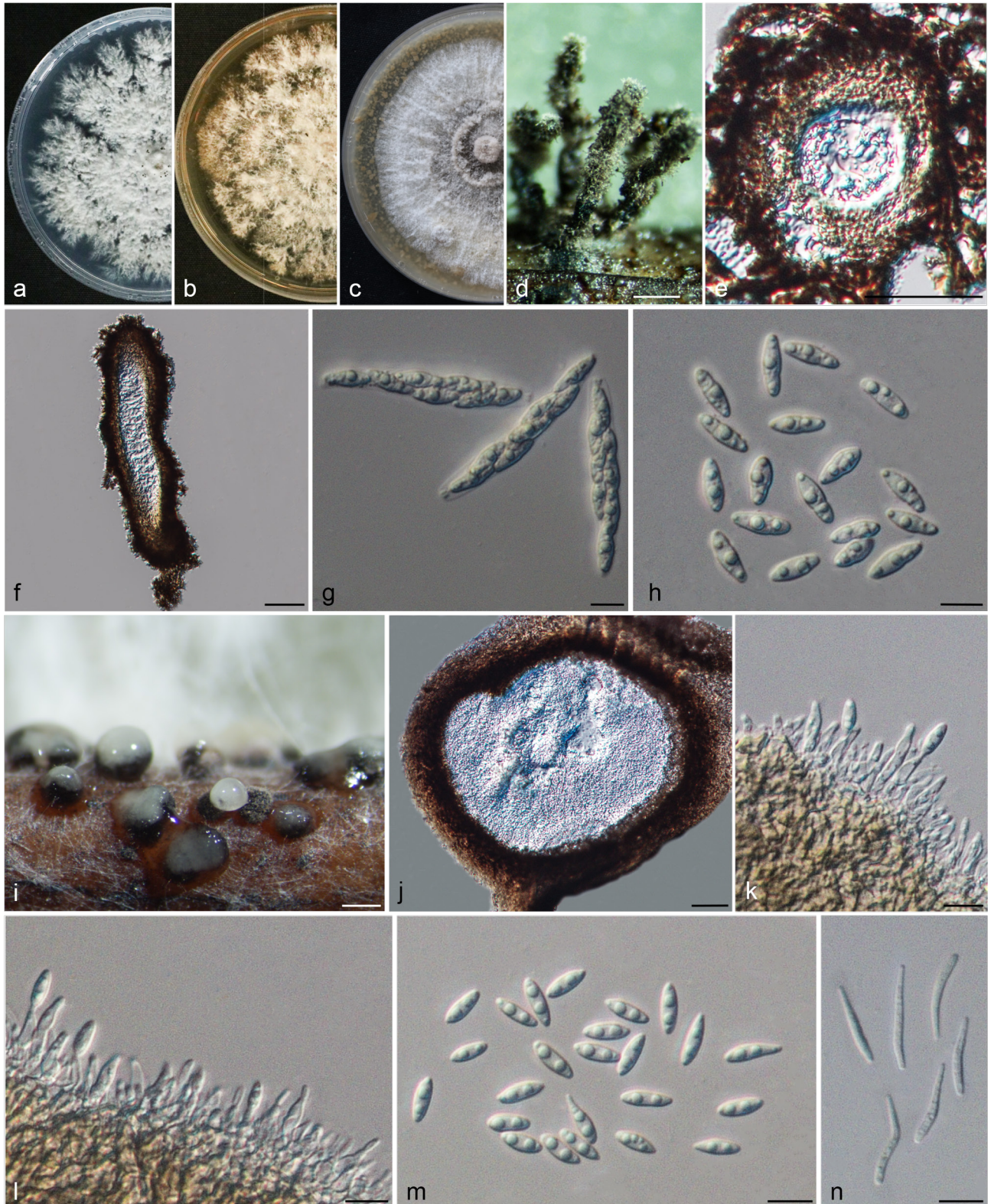


Fig. 7 *Diaporthe sexualispora*. a–c. Colonies on PDA, MEA and OA, respectively; d. ascomata on PNA; e, f. section view of ascomata; g. asci; h. ascospores; i. conidiomata sporulating on PNA; j. section view of conidioma; k, l. conidiophores; m. alpha conidia; n. beta conidia. — Scale bars: d, i = 400 μ m; e, f, j = 50 μ m; g, h, k–n = 10 μ m.

ponkan compared to the length of lesions produced on *C. paradisi*. On *C. reticulata* cv. ponkan, two isolates of *D. citri* caused the largest lesions (mean lesion length > 4 cm), followed by isolate ZJUE 0400 of *D. hubeinensis* and isolate ZJUE 0416 of *D. citriasiatica* (2 cm < mean lesion lengths < 4 cm), and the remaining isolates induced shorter lesions (mean lesion lengths < 2 cm). No discoloured lesions were produced on the shoots inoculated with PDA plugs as controls (Fig. 8, 9).

Representative isolates were selected for inoculation in vivo. These isolates were chosen from isolates exhibiting different aggressiveness based on the in vitro pathogenicity results on *C. paradisi* and *C. reticulata* cv. ponkan. These isolates included ZJUE 0254 and ZJUE 0413 (*D. citri*), ZJUE 0393 and ZJUE 0400 (*D. hubeinensis*), ZJUE 0217 and ZJUE 0416 (*D. citriasiatica*), and ZJUE 0418 (*D. sexualispora*) that showed moderate to high in vitro pathogenicity; and ZJUE 0265 and ZJUE 0372 (*D. jishouensis*), ZJUE 0363 and ZJUE 0394 (*D. penetriteum*)

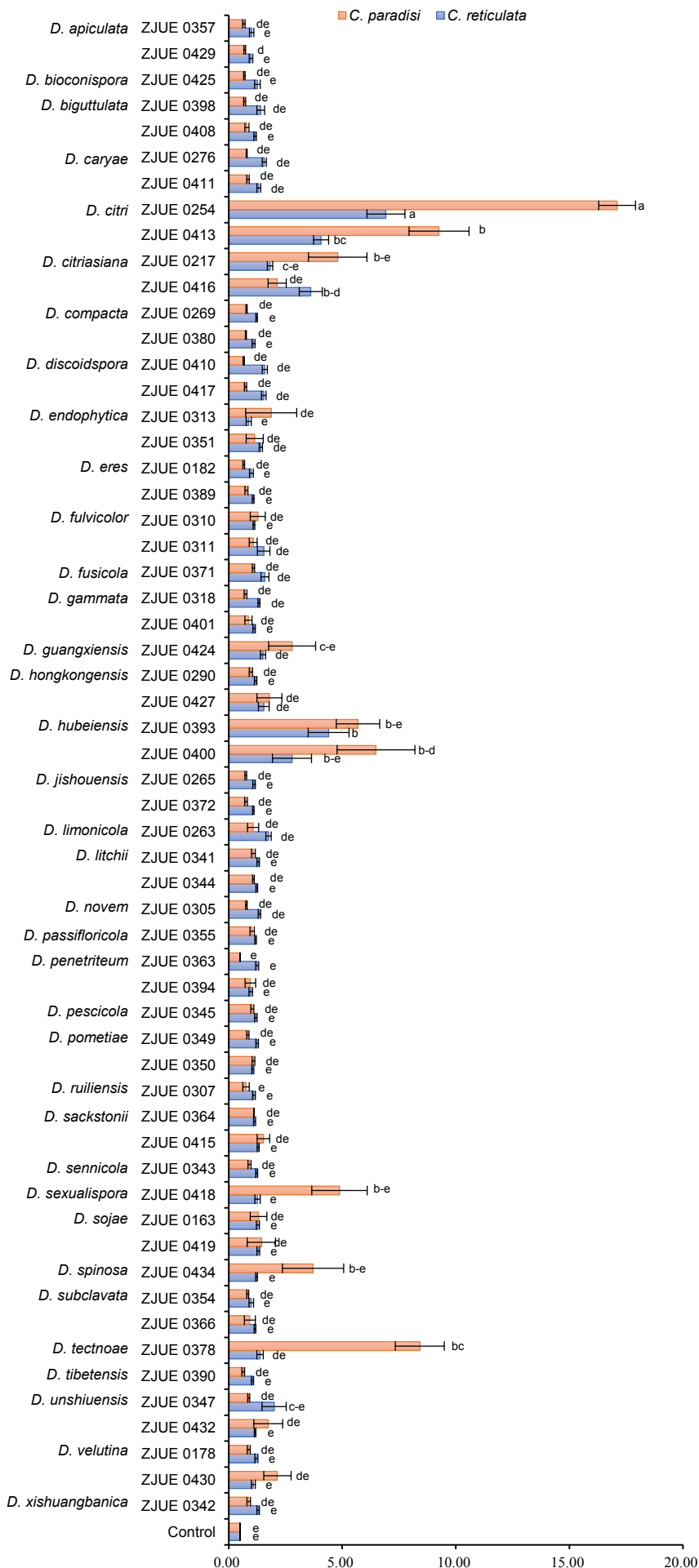


Fig. 8 Mean lesion lengths on *C. paradisi* and *C. reticulata* cv. ponkan shoots inoculated with isolates in *Diaporthe* in vitro after 12 d. Bars represent standard errors. Columns with different letters indicate significant differences according to LSD test with confidence level $\alpha = 0.05$. Control: PDA plugs.

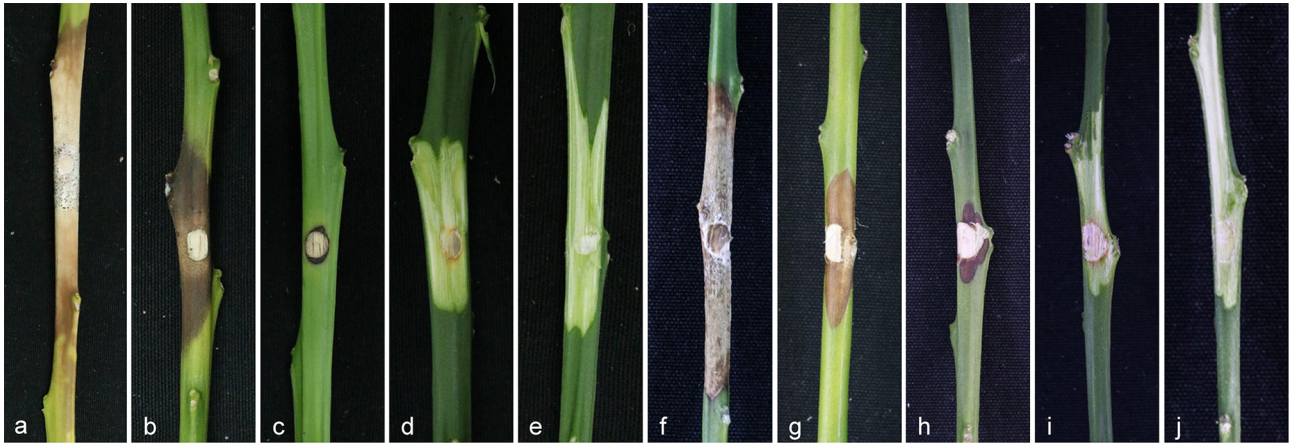


Fig. 9 Pathogenicity test results of inoculated isolates of *Diaporthe* in *C. paradisi* (a–e) and *C. reticulata* cv. ponkan (f–j) shoots 12 d post inoculation. a. Isolate ZJUE 0254 of *D. citri* produced longer lesion length (mean lesion length > 9 cm); b. isolate ZJUE 0217 of *D. citriasiana* produced moderate lesion length (4 cm < mean lesion length < 9 cm); c, d. isolate ZJUE 0355 of *D. passifloricola* and ZJUE 0347 of *D. unshiuensis* produced short lesion lengths or only caused slight discoloration around the inoculated points (mean lesion length < 4 cm); e, j. no lesions were produced on the shoot inoculated with PDA plug; f. isolate ZJUE 0393 of *D. hubeiensis* caused larger lesion (mean lesion length > 4 cm); g. isolate ZJUE 0347 of *D. unshiuensis* caused moderate lesion length (2 cm < mean lesion length < 4 cm); h, i. isolate ZJUE 0398 of *D. biguttulata* and ZJUE 0311 of *D. fulvicolor* caused short lesion lengths or only caused slight discoloration around the inoculated points (mean lesion length < 2 cm).

that showed weak in vitro pathogenicity. After 10 d post inoculation, isolates ZJUE 0254 and ZJUE 0416 (*D. citri*), ZJUE 0393 and ZJUE 0400 (*D. hubeiensis*) produced obvious lesions on all the tested varieties, and caused gummosis on shoots of *C. limon* and citrus hybrid cv. cocktail grapefruit. The symptoms were similar to those observed in the field. Isolates of *D. citriasiana* exhibited moderate to high pathogenicity on detached shoots but produced shorter lesions on *F. margarita* and citrus hybrid cv. cocktail grapefruit, and caused slight discoloration around the inoculated points or no necrosis on *C. limon*. The remaining isolates caused only slight discoloration or no lesions developed on the shoots of all tested varieties. In parallel, no necrosis symptoms were observed on the control shoots. Interestingly, we found that *Diaporthe* isolates rapidly produced

conidiomata at the point of inoculation in live shoots, even for those weakly pathogenic isolates (Fig. 10).

Based on the pathogenicity tests on shoots, six isolates showing moderate to high aggressiveness were selected for inoculation on leaves of *C. tangerina* cv. Hongjv seedlings and plants of *C. limon* and *C. sinensis*. These isolates included ZJUE 0217 and ZJUE 0416 (*D. citriasiana*), ZJUE 0393 and ZJUE 0400 (*D. hubeiensis*), and ZJUE 0254, with ZJUE 0413 (*D. citri*) used as positive control. Ten days post inoculation, only isolates of *D. citri* induced melanose symptoms in leaves (Fig. 11). All isolates inoculated in shoots and on leaves were re-isolated successfully from these lesions. As expected, no isolate of *Diaporthe* was obtained from the negative control inoculations.

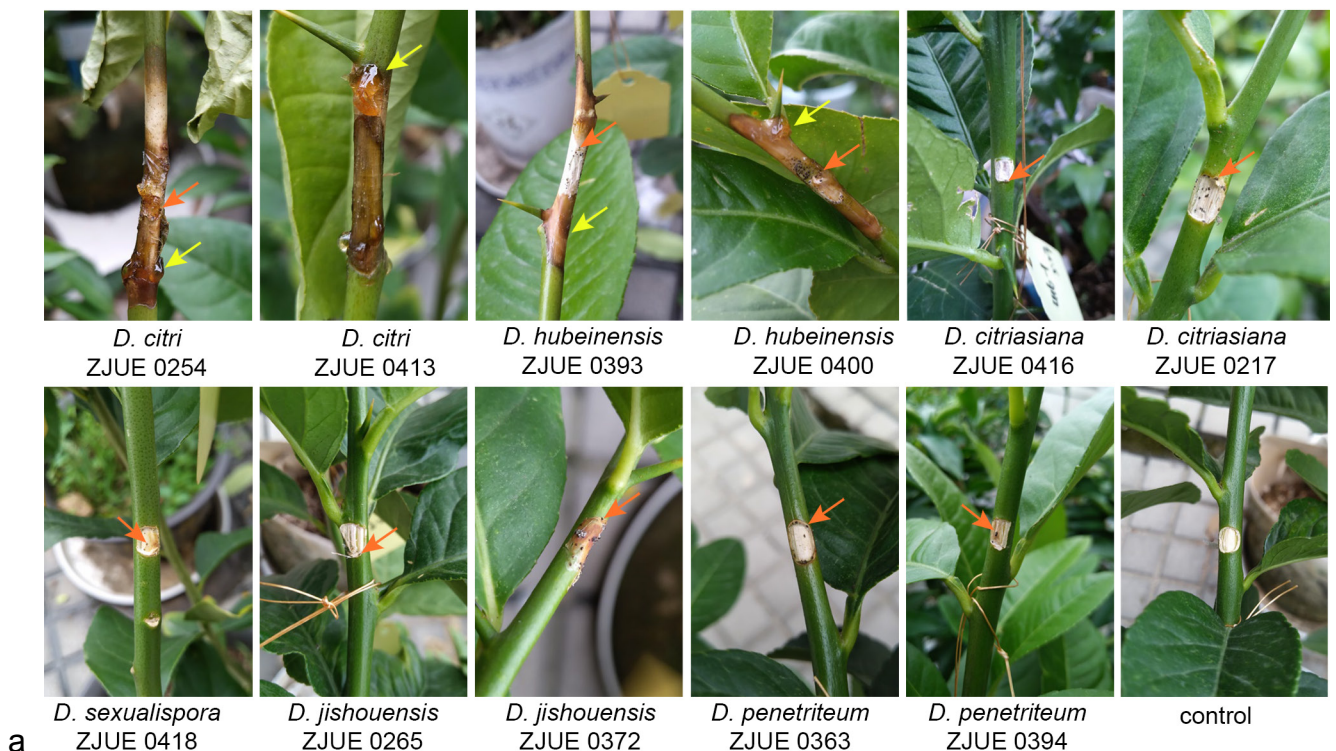


Fig. 10 Symptoms developed in shoots of: a. *C. limon*; b. citrus hybrid cv. cocktail grapefruit; c. *F. margarita*. With isolates of *Diaporthe* 10 d after inoculation. Yellow arrows indicate gum exudate and red arrow indicates a conidioma.



Fig. 10 (cont.)

DISCUSSION

In this study, 1287 *Diaporthe* isolates were obtained from *Citrus* showing symptoms of melanose on leaves, shoots and fruits, dieback, local necrosis and gummosis, canker or wood decay of twigs, branches and trunks based on large-scale surveys in the main citrus production regions in China. Based on multi-locus phylogenetic analyses and morphological features, these isolates were identified to represent 36 species of *Diaporthe*, including 32 known and four novel species. Of the 32 known

species reported here, 14 species are reported on *Citrus* for the first time and two are newly reported from China (Table 1). Our results revealed significant diversity of *Diaporthe* species associated with *Citrus* in China.

Among the *Diaporthe* species obtained, *D. citri* was the most frequently isolated, representing 71.7 % of the isolates in diseased branch samples and 86.5 % in melanose samples. In addition, the pathogenicity tests on citrus leaves and shoots demonstrated that *D. citri* was more aggressive to *Citrus* than other species of *Diaporthe*. Therefore, we rank *D. citri* as



Fig. 11 Melanose symptom induced by *D. citri* isolate (ZJUE 0254) 10 d after inoculation on: a. *C. tangerina* cv. Hongjv; b. *C. sinensis*; c. after 3 d inoculation on *C. limon* leaves.

the dominant pathogen of the genus *Diaporthe* on *Citrus* in China, which is consistent with previous research (Huang et al. 2013, Chaisiri 2018). A recent study showed that *D. citri* has frequent sexual reproduction in orchards (Xiong et al. 2021). The occurrence of sexual reproduction is more suitable for the long-term survival of fungi and increasing their adaptability to changing environments (Nieuwenhuis & James 2016, Drenth et al. 2019). However, comparative population genetic studies of other *Diaporthe* species are needed to understand the potential ecological factors responsible for the dominance of *D. citri* over other *Diaporthe* species in China.

Aside from the highly virulent isolates of *D. citri*, isolates of *D. hubeiensis* moderately aggressive on citrus shoots, and could not cause melanose on leaves. In this study, only two isolates of *D. hubeiensis* were recovered from leaf melanose of *C. reticulata* and *C. sinensis*. *Diaporthe hubeiensis* was first reported in the diseased trunk of *Vitis vinifera* in Hubei Province of China. A pathogenicity test showed that *D. hubeiensis* was the least aggressive compared to other *Diaporthe* species obtained from diseased grapevines (Manawasinghe et al. 2019), which is contrary to the pathogenicity results on citrus in this study. Pathogenicity results indicated that *D. hubeiensis* could cause moderate length lesions in citrus shoots in vitro, shoot dieback and gummosis on lemon and cocktail grapefruits plants, and wither-tip on kumquats plants. However, the number of isolates collected in this study was small, and more isolates will need to be obtained to evaluate their risk to *Citrus*.

Pathogenicity tests showed that the majority of the *Diaporthe* species obtained in this study are weakly aggressive or non-aggressive to citrus shoots. Species of *Diaporthe* have endophytic, saprophytic and pathogenic lifestyles. Some *Diaporthe* fungi can be both endophytic and pathogenic, depending on the host species and the health status of the host plant. For example, Guarnaccia & Crous (2017) reported that *D. limonicola* causes cankers on lemon branches, while Dong et al. (2021) isolated this fungal species from healthy leaves of *C. grandis* cv. Tomentosa. Gao et al. (2016) isolated the same *Diaporthe* species from leaves of both symptomatic and asymptomatic *Camellia sinensis*, suggesting that this species might be a latent pathogen. In this study, several *Diaporthe* species were isolated from diseased tissues, which had previously been reported from healthy citrus tissues (Huang et al. 2015) (Table 1). In addition, weak or non-pathogenic *Diaporthe* isolates were isolated from branch cankers in this study, while other fungal taxa, such as *Lasiodiplodia*, *Colletotrichum* and *Alternaria* were also isolated from the same samples. Thus, we speculate that the strains of less aggressive *Diaporthe* species that were isolated from diseased tissues could be latent pathogens. They occur in healthy citrus trees under normal growth conditions, but can transform into pathogens when the host is weakened or experiencing stress conditions, and continue to survive as saprophytes in decaying plant tissues.

Previous studies have reported the presence of multiple *Diaporthe* spp. in *Citrus* (Table 1), and the results of this study further support these findings. Indeed, more novel species will likely be found in the future. Several reasons may have contributed to the high species diversity of *Diaporthe* on *Citrus*. Firstly, our survey covered the broadest geographic and *Citrus* host range reported thus far from China. Secondly, many of the isolates obtained in this study are weakly aggressive or non-pathogenic and likely represent endophytes or latent pathogens. Previous studies have suggested significant undiscovered diversity among endophytic fungi (Arnold et al. 2000, Botella & Diez 2011). Thirdly, several species in *Diaporthe* have a wide host range and can move between hosts among geographic regions. For example, *D. unshiuensis* was originally reported on *Citrus* in China (Huang et al. 2015), but the fungus has also been found on *Vitis* (Manawasinghe et al. 2019) and *Pyrus* spp. (Guo et al. 2020) in China. Extended surveys over multiple years (2017–2020) helped us reveal that some of these *Diaporthe* species likely migrated from non-citrus host plants. These three factors are not mutually exclusive, and all could contribute to the high *Diaporthe* species diversity observed in this study. Additional research on *Diaporthe* from non-citrus plants in orchards adjacent to citrus plants are needed to test the third possibility. In addition to the three reasons mentioned above, the high number of *Diaporthe* species on *Citrus* may also be due to the changing criteria for species delimitation. Recently, Hilário et al. (2021) used genealogical concordance and coalescent-based species delimitation to assess species boundaries in the *D. eres* complex and identified that the *D. eres* complex as a single species, suggesting that the identification of species in *Diaporthe* has been largely overestimated. Therefore, a coherent approach consisting of genealogical concordance criteria and methods for detecting recombination should be implemented, or methods based on whole-genome sequence analyses developed in the future to circumscribe species in the genus *Diaporthe* (Xu 2020).

In conclusion, results of this study present a large-scale investigation into 36 species of *Diaporthe* associated with *Citrus* in China. Overall, *D. citri* was the most prevalent and aggressive species causing citrus branch diseases and melanose. Among the 36 species, we found a range of frequency and pathogenicity on citrus, consistent with a diversity of lifestyles and associations between *Diaporthe* species and *Citrus* in China. Based on previous reports and the results of this study (Table 1), there are currently 59 species of *Diaporthe* associated with *Citrus*. Additional novel species will likely be found in the future. The speciation of these species, the potential hybridization among them, and their tissue and host ranges are all worthy of further in-depth research and exploration. The DNA sequence polymorphisms among species identified here will enable the development of rapid molecular diagnosis of citrus diseases caused by *Diaporthe*. Together, our increased understanding

of the diversity of *Diaporthe* pathogens, their pathogenicity, and potential rapid diagnosis and monitoring tools provide a solid foundation for accurate assessments of the threats of this group of pathogens to citrus productions in China (Xu 2022). Such assessments will be essential for developing targeted prevention and management strategies against the *Diaporthe* diseases in citrus among geographic regions.

Acknowledgements This research was supported by the Key Research and Development Program of Zhejiang Province (2019C02022), the National Key Research and Development Program (2017YFD0202000) and the China Agriculture Research System (CARS-26). We thank Siqing Zhao, Dekuan Ding, Qianbin Huang, Weilong Li, Jimiao Peng, Zhenhua Tan, Tingmi Yang, Ziqin Bai, Fei Jiang, Zhanxu Pu, Zhongdong Hu, Zimin Zhao, Youli You, Shansheng Tan, Dehe Li and Lan Cheng for their assistance during sample collections. We thank Xin Wang and Lixin Wu for providing the citrus shoots for pathogenicity tests. We thank Xiaoxiao Feng from the agricultural experiment station of Zhejiang University for her assistance during the pathogenicity tests in vivo.

Declaration on conflict of interest The authors declare that there is no conflict of interest.

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Supplementary material

Fig. S1 Phylogenetic tree generated by Bayesian inference analyses based on the individual *cal*, *his3*, *tef1* and *tub2* (a–d) sequence alignment of species in *D. eres* species complex. Bayesian posterior probability (PP \geq 0.9) and IQtree bootstrap support values (ML \geq 50 %) were presented above branches as follows: PP/ML. PP < 0.9 and ML < 50 % of branches marked with *, and absent marked with -. Ex-type strains in **bold**. The tree is rooted to *Diaporthe citri* (CBS 135422). The clades marked with colour blocks contain isolates of *Diaporthe* obtained in this study.

Fig. S2 Phylogenetic tree generated by Maximum likelihood (a) and Bayesian inference analyses (b–e) based on the individual ITS, *cal*, *his3*, *tef1* and *tub2* sequence alignment of species in *Diaporthe* spp. Bayesian posterior probability (PP \geq 0.9) and IQtree bootstrap support values (ML \geq 50 %) were presented above branches as follows: PP/ML, except for ITS gene tree, which only presented IQtree bootstrap support values. PP < 0.9 and ML < 50 % of branches marked with *, and absent marked with -. Ex-type strains in **bold**. The tree is rooted to *Diaporthe citri* (CBS 135422). The clades marked with colour blocks contain isolates of *Diaporthe* obtained in this study.

Table S1 Details of all *Diaporthe* isolates obtained in this study.