

# Botryosphaerialean fungi associated with woody oil plants cultivated in Sichuan Province, China

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## Abstract

Woody oil plants are important economic trees which are widely cultivated and distributed throughout China. Surveys conducted during 2020 and 2021 on several woody oil plantations from five regions of Sichuan Province, China, revealed a high diversity of Botryosphaerialean fungi. The identification of 50 botryosphaeriaceous isolates was carried out based on both morphology and multi-gene phylogenetic analysis of internal transcribed spacer region (ITS), translation elongation factor 1-alpha gene (*tef1*) and  $\beta$ -tubulin gene (*tub2*). This allowed the identification of twelve previously known Botryosphaeriales species: *Aplosporella prunicola*, *A. ginkgonis*, *Barriopsis tectonae*, *Botryosphaeria dothidea*, *Bo. fabicerciana*, *Diplodia mutila*, *Di. seriata*, *Dothiorella sarmentorum*, *Neofusicoccum parvum*, *Sardiniella guizhouensis*, *Sphaeropsis citrigena*, and *Sp. guizhouensis*, and four novel species belonging to the genera *Diplodia* and *Dothiorella*, viz. *Di. acerigena*, *Di. pistaciicola*, *Do. camelliae* and *Do. zanthoxyli*. The dominant species isolated across the surveyed regions were *Botryosphaeria dothidea*, *Sardiniella guizhouensis* and *Diplodia mutila*, representing 20%, 14% and 12% of the total isolates, respectively. In addition, most isolates were obtained from *Pistacia chinensis* (14 isolates), followed by *Camellia oleifera* (10 isolates). The present study enhances the understanding of Botryosphaeriales species diversity on woody oil plants in Sichuan Province, China.

## Keywords

Botryosphaeriales, diversity, new species, phylogeny, taxonomy

## Introduction

Botryosphaeriaceae is a diverse group of fungi that includes endophytes, saprobes and plant pathogens (Phillips et al. 2013). They have broad host ranges, and are widely distributed in tropical and temperate regions (Batista et al. 2021). Botryosphaeriaceae was introduced by Theissen and Sydow (1918) to accommodate three genera *Botryosphaeria*, *Dibotryon* and *Phaeobotryon*. Botryosphaeriales was proposed to include the single family, Botryosphaeriaceae, based on multi-gene phylogeny (Schoch et al. 2006). Up to date, six families and 32 genera are accepted in Botryosphaeriales, while Botryosphaeriaceae is known to be the largest monophyletic family, including 22 genera and more than 200 species (<https://botryosphaeriales.org/>, accessed on 15<sup>th</sup> April 2023).

The members of Botryosphaeriaceae have been taxonomically characterized based on both sexual and asexual morphs. The production of large, ovoid to oblong, typically hyaline, aseptate ascospores, which may become brown and septate with age, as well as bitunicate asci within unilocular or multilocular botryose ascomata known as pseudothecia is typical to the sexual state (Sivanesan 1984; Phillips et al. 2005). The asexual states of Botryosphaeriaceae exhibit a wide range of conidial morphologies; for example, its conidia can be thin-walled and hyaline, or thick-walled and pigmented, aseptate or 1–2-septate (Phillips et al. 2005). Additionally, the spermatial states were also frequently observed in Botryosphaeriaceae species, which produced unicellular, hyaline, allantoid to rod-shaped spermatia on culture. Botryosphaeriaceae species are significantly different from other fungi in that the color of its aerial hyphae, changing from gray to black with age on 2% potato dextrose agar (PDA), which can be used for the rapid determination of botryosphaeriaceous fungi.

The geographic distribution and host range of botryosphaeriaceous taxa are diverse. Seven genera in Botryosphaeriaceae: *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neodeightonia*, *Neofusicoccum* and *Phaeobotryon* are common and frequently reported from various geographical regions (Batista et al. 2021), while *Botryobambusa*, *Oblongocollomyces*, *Sakireeta* and *Sardiniella* appear to be limited to a single region or country (Liu et al. 2012; Crous et al. 2015; Linaldeddu et al. 2016; Yang et al. 2017; Dissanayake et al. 2021). Many Botryosphaeriaceae species have wide host ranges (e. g. *Botryosphaeria dothidea*, *Diplodia mutila*, *Dothiorella sarmentorum*, *Lasiodiplodia theobromae* and *Neofusicoccum parvum*), while other species have narrower host ranges (e. g. *Diplodia olivarum* was reported on olive, oleaster, carob, grapevine, almond et al.) (Lazzizera et al. 2008; Granata et al. 2011; Linaldeddu et al. 2015; Olmo et al. 2016) or even in very specific hosts (e. g. *Eutiarosporella darliae* was only reported on infected wheat and wheat-stubble) (Thynne et al. 2015; Farr and Rossman 2021). Different species of Botryosphaeriaceae exhibit different environmental adaptations and host preferences (Braunsdorf et al. 2016). Botryosphaeriaceous taxa

with narrow host ranges or limited geographic distribution will be more susceptible to climatic effects (Slippers et al. 2017; Li et al. 2020).

Woody oil plants are economically important as they are used for the production of cooking and industrial oil. Recently, many Botryosphaeriaceae species have been frequently reported on woody oil plants. *Diplodia olivarum* was first reported from rotting olive drupes in Italy (Lazzizzera et al. 2008) and later it was reported as associated with declining *Prunus dulcis* trees in Spain (Gramaje et al. 2012). *Diplodia insularis* was isolated from branch canker of *Pistacia lentiscus* in Italy (Linaldeddu et al. 2015). *Dothiorella gregaria* was isolated from the stems with asymptomatic of *Zanthoxylum bungeanum* in China (Li et al. 2016b). *Botryosphaeria dothidea*, *Diplodia mutila*, *Di. seriata*, *Dothiorella iberica*, *Do. omnivora*, *Do. sarmentorum*, *Lasioidiplodia citricola*, *L. pseudotheobromae*, *L. theobromae*, *Neofusicoccum mediterraneum*, *N. nonquaesitum*, *N. parvum*, *N. ribis*, *N. vitifusiforme*, and *Neoscytalidium dimidiatum* have been reported as pathogens of *English walnut* (*Juglans regia* L.) in California (Chen et al. 2014), Chile (Jimenez Luna et al. 2022), China (Li et al. 2016a; Zhang et al. 2017), Iran (Abdollahzadeh et al. 2013; Panahandeh et al. 2019), South Africa (Cloete et al. 2011), Spain (Gramaje et al. 2012) and USA (Chen et al. 2014). However, very little is known about the Botryosphaeriales species occurring on native woody oil plants in China. Hence, the aim of this study was to gain a more comprehensive understanding of the diversity of Botryosphaeriaceae species associated with common woody oil plants grown in Sichuan Province, China.

## Materials and methods

### Isolates and morphology

The isolates in this study were collected from the woody oil tree plantations in Sichuan Province during the period of 2020 and 2021. The hosts include *Acer truncatum*, *Camellia oleifera*, *Idesia polycarpa*, *Olea europaea*, *Paeonia suffruticosa*, *Pistacia chinensis*, *Vernicia fordii* and *Zanthoxylum bungeanum*. The samples were collected from decayed stems, branches and twigs of woody oil trees. Mature fruiting bodies were selected for fungal isolation and for morphological observations under stereo microscope Motic SMZ 168 series. Measurements were made with Tarosoft Image Frame Work program v. 0.9.7 (Liu et al. 2010). Thirty conidia/ascospores were measured per isolate, and 10–30 measurements were taken of other morphological structures. At least 20 conidia/ascospores were used to calculate the average length/width ratio (L/W). Single spore isolation was conducted in accordance with the methods described in Chomnunti et al. (2014). Germinated spores were individually placed on PDA plates and grown at 25 °C in daylight.

Herbarium specimens were stored in the herbarium of Cryptogams Kunming Institute of Botany, Academia Sinica (KUN-HKAS) and duplicated at Herbarium, University of Electronic Science and Technology (**HUEST**), Chengdu, China. Living cultures were deposited at China General Microbiological Culture Collection Centre (**CGMCC**), Beijing, China and duplicated at the University of Electronic Science and Technology Culture Collection (**UESTCC**), Chengdu, China. MycoBank numbers were registered as outlined in MycoBank (<http://www.MycoBank.org>. Accessed on 11<sup>th</sup> November 2022).

## DNA extraction, PCR amplification and sequencing

The total genomic DNA was extracted from 7day-old isolates grown on 2% PDA median at 25 °C, using the EZ gene<sup>TM</sup> fungal gDNA kit (GD2416), following the manufacturer's instructions and protocols. Partial gene sequences were determined for the internal transcribed spacer 1 and 2 including the intervening 5.8S nrDNA gene (ITS), the nuclear ribosomal 28S large subunit (LSU), the translation elongation factor 1- $\alpha$ -phagene (*tef1*), and the  $\beta$ -tubulin gene (*tub2*). The primers used for amplification are ITS5/ITS4 for ITS (White et al. 1990), LR0R/LR5 for LSU (Vilgalys and Hester 1990), EF1-728F/EF1-986R for *tef1* (Carbone and Kohn 1999) and Bt2a/Bt2b for *tub2* (Glass and Donaldson 1995). Polymerase chain reaction (PCR) amplification conditions were followed as of Dissanayake et al. (2021). PCR products were sent for sequencing at Beijing Tsingke Biological Engineering Technology and Services Co. Ltd. (Beijing, P.R. China). All newly generated sequences are deposited in GenBank, and the obtained accession numbers are listed in Table 1.

## Phylogenetic analyses

Sequence data for phylogenetic analyses were obtained from GenBank and from recent publications regarding Botryosphaeriaceae fungi (Dissanayake et al. 2021; Xiao et al. 2021; Zhang et al. 2021; Rathnayaka et al. 2022) (See Suppl. material 1). The single gene alignments were performed using MAFFT v.7.429 online service (<https://mafft.cbrc.jp/alignment/server/>, accessed on 15 October 2022) (Kato et al. 2019) and ambiguous regions were excluded using TrimAI with the option “-automated1”, which trimmed sequences based on similarity statistics (Capella-Gutiérrez et al. 2009). Multi-gene sequences were concatenated by Sequence matrix software (Vaidya et al. 2011). Multi-gene phylogenetic analyses were obtained from maximum likelihood (ML) and Bayesian inference (BI) analyses following Dissanayake et al. (2020).

ML analyses was performed using RAxML (Stamatakis 2006). The tree search included 1,000 non-parametric bootstrap replicates and the best scoring tree was selected from suboptimal trees under the GTRGAMMA substitution model. Maximum

**Table 1.** All newly generated sequences in this study. Ex-type strains are indicated with \*. N/A: Not available.

| Taxon                           | Stain Number   | GenBank Accession Number |             |             |
|---------------------------------|----------------|--------------------------|-------------|-------------|
|                                 |                | ITS                      | <i>tef1</i> | <i>tub2</i> |
| <i>Aplosporella ginkgonis</i>   | UESTCC 22.0091 | OQ190504                 | OQ241438    | N/A         |
| <i>Aplosporella prunicola</i>   | UESTCC 22.0090 | OQ190505                 | N/A         | N/A         |
| <i>Barriopsis tectonae</i>      | UESTCC 22.0092 | OQ190506                 | OQ241439    | N/A         |
| <i>Botryosphaeria dothidea</i>  | UESTCC 22.0111 | OQ190507                 | OQ241440    | N/A         |
|                                 | UESTCC 22.0109 | N/A                      | OQ241441    | N/A         |
|                                 | UESTCC 22.0112 | OQ190508                 | OQ241442    | N/A         |
|                                 | UESTCC 22.0113 | OQ190509                 | OQ241443    | N/A         |
|                                 | UESTCC 22.0108 | OQ190510                 | OQ241444    | N/A         |
|                                 | UESTCC 22.0116 | OQ190511                 | OQ241445    | N/A         |
|                                 | UESTCC 22.0114 | OQ190512                 | OQ241446    | N/A         |
|                                 | UESTCC 22.0115 | OQ190513                 | OQ241447    | N/A         |
|                                 | UESTCC 22.0110 | OQ190514                 | OQ241448    | N/A         |
|                                 | UESTCC 22.0107 | OQ190515                 | OQ241449    | N/A         |
|                                 | UESTCC 22.0117 | OQ190516                 | OQ241450    | N/A         |
|                                 | UESTCC 22.0118 | OQ190517                 | OQ241451    | N/A         |
| <i>Diplodia acerigena*</i>      | CGMCC 3.24157  | OQ190518                 | OQ241452    | N/A         |
| <i>Diplodia acerigena</i>       | UESTCC 22.0074 | OQ190519                 | OQ241453    | OQ338163    |
|                                 | UESTCC 22.0075 | OQ190520                 | OQ241454    | OQ338164    |
| <i>Diplodia mutila</i>          | UESTCC 22.0064 | OQ190521                 | OQ241455    | OQ338165    |
|                                 | UESTCC 22.0065 | OQ190522                 | OQ241456    | OQ338166    |
|                                 | UESTCC 22.0069 | OQ190523                 | OQ241457    | OQ338167    |
|                                 | UESTCC 22.0068 | OQ190524                 | OQ241458    | OQ338168    |
|                                 | UESTCC 22.0067 | OQ190525                 | OQ241459    | OQ338169    |
|                                 | UESTCC 22.0063 | OQ190526                 | OQ241460    | OQ338170    |
|                                 | CGMCC 3.24156  | OQ190527                 | OQ241461    | OQ338171    |
| <i>Diplodia pistaciicola *</i>  | UESTCC 22.0071 | OQ190528                 | OQ241462    | OQ275062    |
| <i>Diplodia seriata</i>         | UESTCC 22.0072 | OQ190529                 | OQ241463    | N/A         |
| <i>Dothiorella camelliae</i>    | UESTCC 22.0080 | OQ190530                 | N/A         | OQ275063    |
| <i>Dothiorella camelliae*</i>   | CGMCC 3.24158  | OQ190531                 | OQ241464    | OQ275064    |
| <i>Dothiorella camelliae</i>    | UESTCC 22.0079 | OQ190532                 | OQ241465    | OQ275065    |
|                                 | UESTCC 22.0078 | OQ190533                 | OQ241466    | OQ275066    |
| <i>Dothiorella sarmentorum</i>  | UESTCC 22.0076 | OQ190534                 | N/A         | OQ275067    |
|                                 | UESTCC 22.0077 | OQ190535                 | OQ241467    | OQ275068    |
| <i>Dothiorella zanthoxyli *</i> | CGMCC 3.24159  | OQ190536                 | OQ241468    | OQ275069    |
| <i>Dothiorella zanthoxyli</i>   | UESTCC 22.0083 | OQ190537                 | OQ241469    | OQ275070    |
|                                 | UESTCC 22.0084 | OQ190538                 | OQ241470    | OQ275071    |
| <i>Neofusicoccum parvum</i>     | UESTCC 22.0096 | OQ190539                 | OQ241471    | N/A         |
|                                 | UESTCC 22.0094 | OQ190540                 | N/A         | N/A         |
|                                 | UESTCC 22.0093 | OQ190541                 | N/A         | N/A         |
|                                 | UESTCC 22.0095 | OQ190542                 | N/A         | N/A         |
| <i>Sardiniella guizhouensis</i> | UESTCC 22.0100 | OQ190543                 | OQ241472    | N/A         |
|                                 | UESTCC 22.0101 | OQ190544                 | OQ241473    | N/A         |
|                                 | UESTCC 22.0099 | OQ190545                 | OQ241474    | N/A         |
|                                 | UESTCC 22.0097 | OQ190546                 | OQ241475    | N/A         |
|                                 | UESTCC 22.0098 | OQ190547                 | OQ241476    | N/A         |
|                                 | UESTCC 22.0102 | OQ190548                 | OQ241477    | N/A         |
| <i>Sphaeropsis citrigena</i>    | UESTCC 22.0103 | OQ190549                 | OQ241478    | N/A         |
|                                 | UESTCC 22.0106 | OQ190550                 | OQ241479    | N/A         |
|                                 | UESTCC 22.0105 | OQ190551                 | OQ241480    | N/A         |
| <i>Sphaeropsis citrigena</i>    | UESTCC 22.0104 | OQ190552                 | OQ241481    | N/A         |
| <i>Sphaeropsis guizhouensis</i> |                |                          |             |             |

likelihood bootstrap values equal or greater than 75% are marked near each node of the phylogenetic tree.

Bayesian analyses was performed in MrBayes 3.2.6 (Ronquist et al. 2012). The program MrModeltest 2.2 (Nylander 2004) was used to determine the best nucleotide substitution model for each data partition. The Markov Chain Monte Carlo (MCMC) sampling approach was used to calculate the posterior probabilities (PP) (Rannala and Yang 1996). Bayesian analyses of four simultaneous Markov chains were run for 10,000,000 generations with trees sampled every 1,000<sup>th</sup> generations. The first 20% of trees, representing the burn-in phase of the analyses, were discarded, and the remaining trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree. PP values equal or greater than 0.95 are marked near each node.

Trees were visualized with FigTree v1.4.0 (Rambaut 2006), and the layout was edited using Adobe Illustrator CS6 software (Adobe Systems, USA).

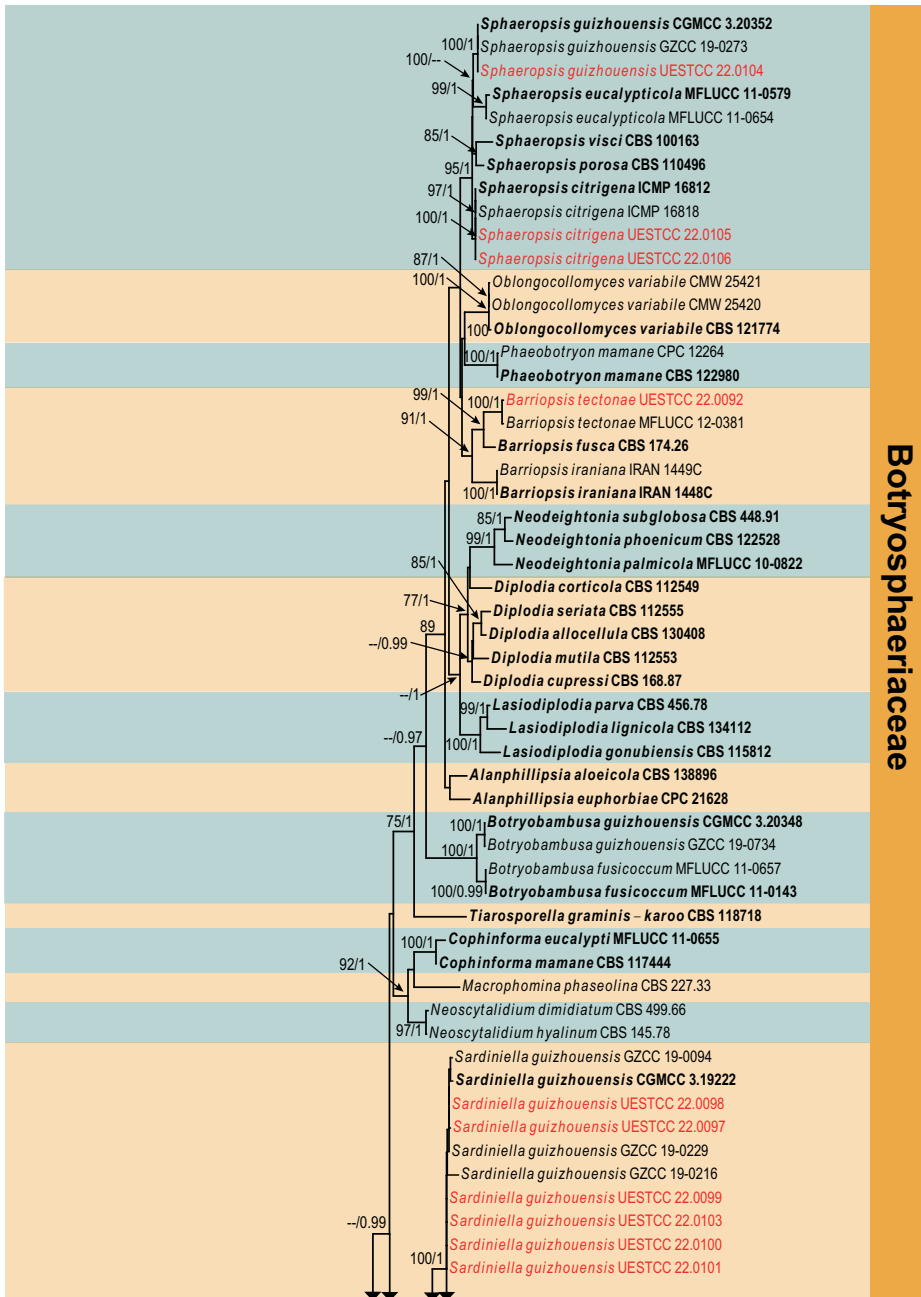
## Results

### Phylogenetic analyses

A concatenated dataset of ITS and *tef1* was used to determine the phylogenetic position of Aplosporellaceae and Botryosphaeriaceae isolates obtained in this study. Combined sequences of ITS and *tef1* were used for the analyses of *Botryosphaeria*, while ITS, *tef1* and *tub2* were used for the analyses of *Diplodia* and *Dothiorella* isolates. All details of the alignments are provided in Table 2.

In an overview phylogenetic tree (Fig. 1), sixteen newly obtained isolates were nested with four genera of Botryosphaeriaceae, representing seven known species viz. *Barriopsis tectonae*, *Neofusicoccum parvum*, *Sardiniella guizhouensis*, *Sphaeropsis guizhouensis* and *Sp. citrigena*. Two isolates were clustered with the genus *Aplosporella* (Aplosporellaceae), and were identified as *A. ginkgonis* and *A. prunicola*.

Three individual phylogenetic trees were constructed for the genera *Botryosphaeria*, *Diplodia* and *Dothiorella*. Twelve isolates belonged to the genus *Botryosphaeria* and ten of them were nested with *Bo. dothidea*, while the remaining two isolates clustered with *Bo. fabicerciana* (Fig. 2). Another twelve isolates were treated in *Diplodia* and seven isolates were clustered with two known species of *Diplodia* (*Di. mutila* and *Di. seriata*, Fig. 3). The other five isolates did not cluster with any previously known *Diplodia* species, thus, two novel species were preliminarily identified based on phylogenetic evidence. Eight isolates were nested within *Dothiorella* and six isolates of them were occupied in the basal position of the *Dothiorella* tree and formed two well-supported subclades, representing two new species. The other two isolates were nested within the *Do. sarmentorum* isolates (Fig. 4).



**Figure 1.** Phylogram generated from RAxML analysis based on combined ITS and *tef1* sequence data of *Botryosphaeriaceae* and *Aplosporellaceae* isolates. The tree was rooted to *Lecanosticta acicula* (LNPV 252). The ML ( $\geq 75\%$ ) and BI ( $\geq 95\%$ ) bootstrap supports are given near the nodes, respectively. Isolates from this study are marked in red and ex-type strains are marked in bold.

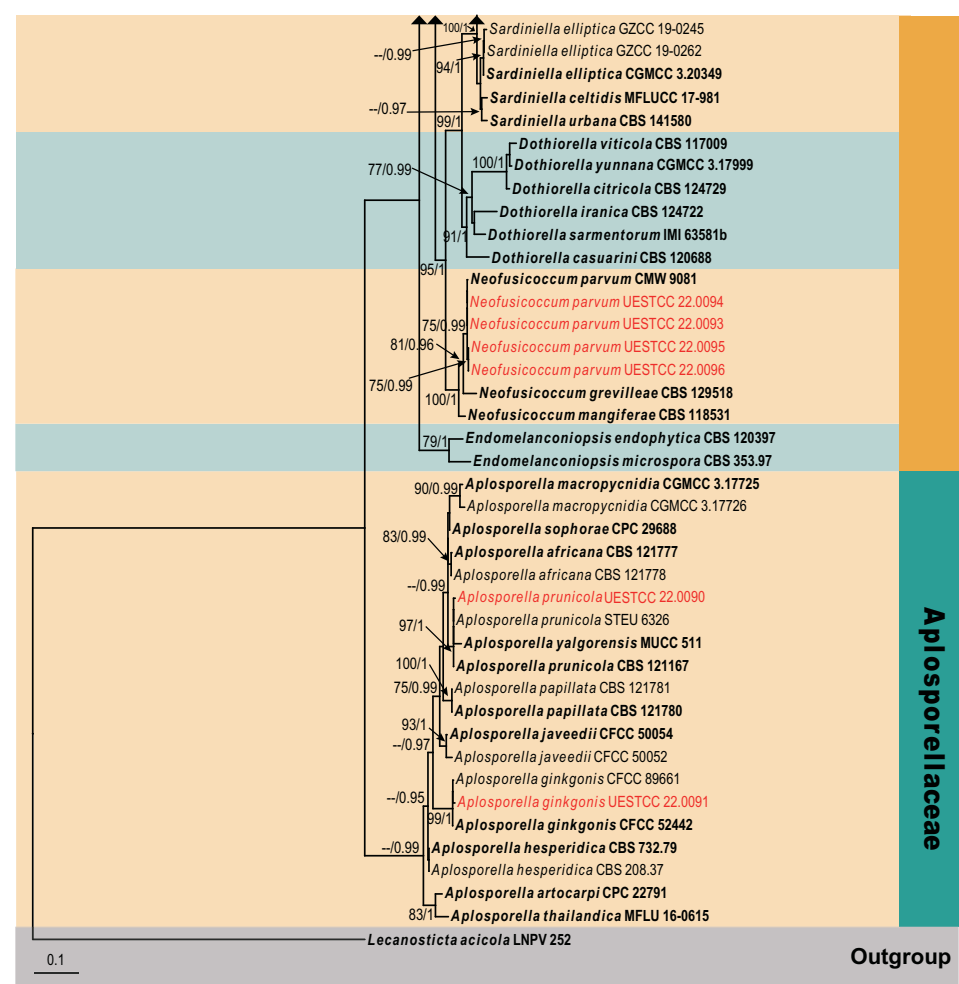
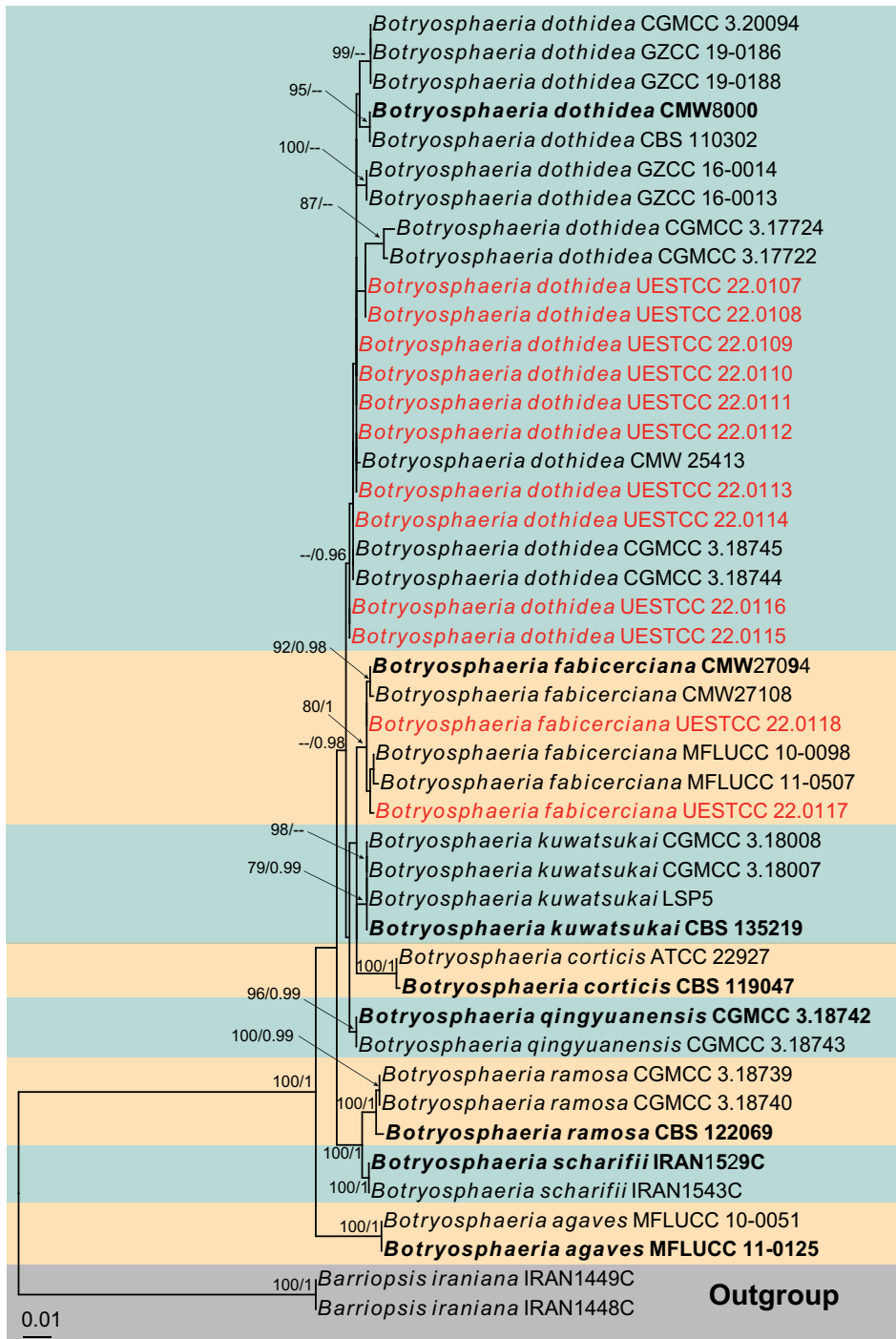


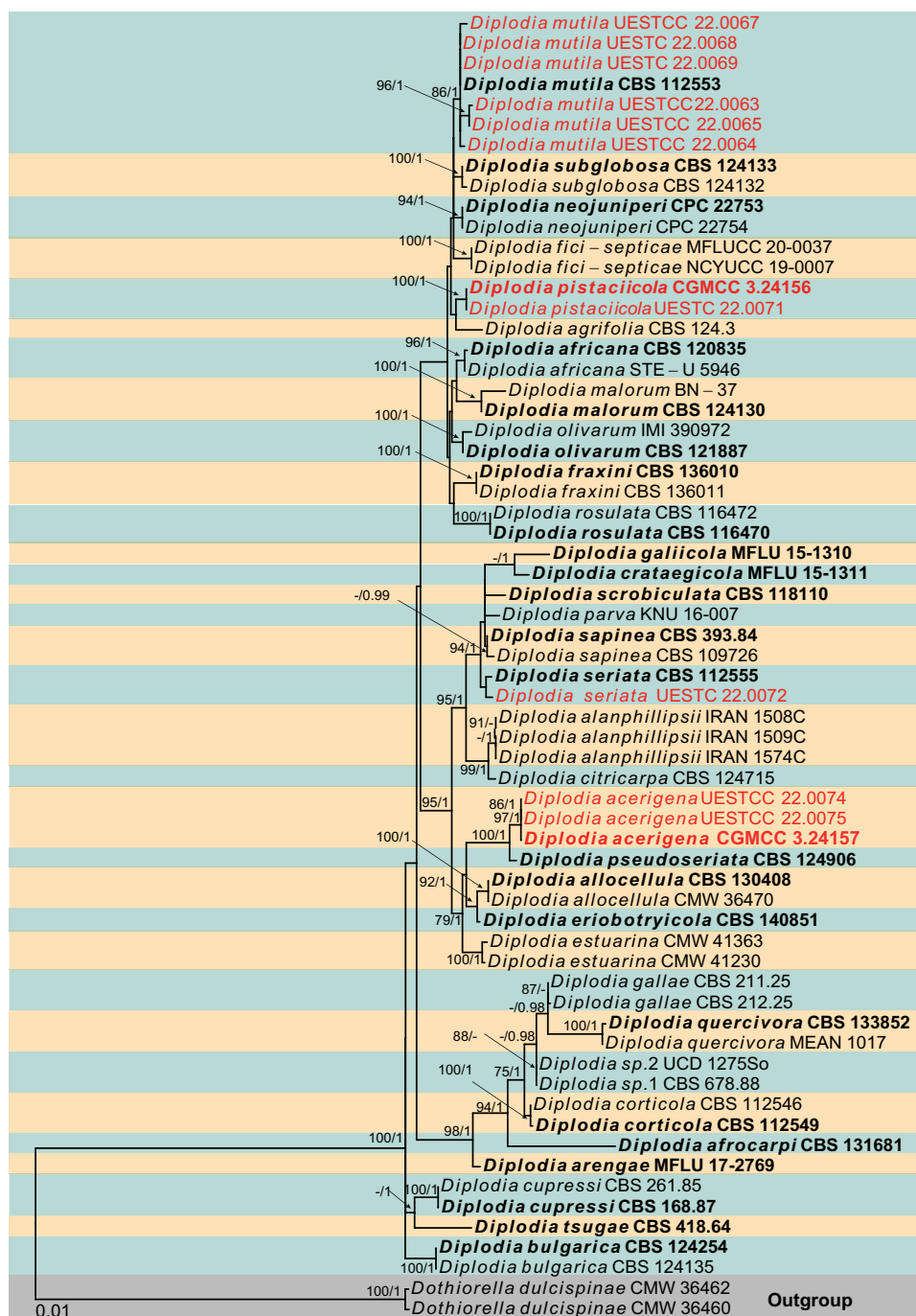
Figure 1. Continued.

Table 2. Alignment details and ML, BI analyses results of each phylogenetic tree constructed in this study.

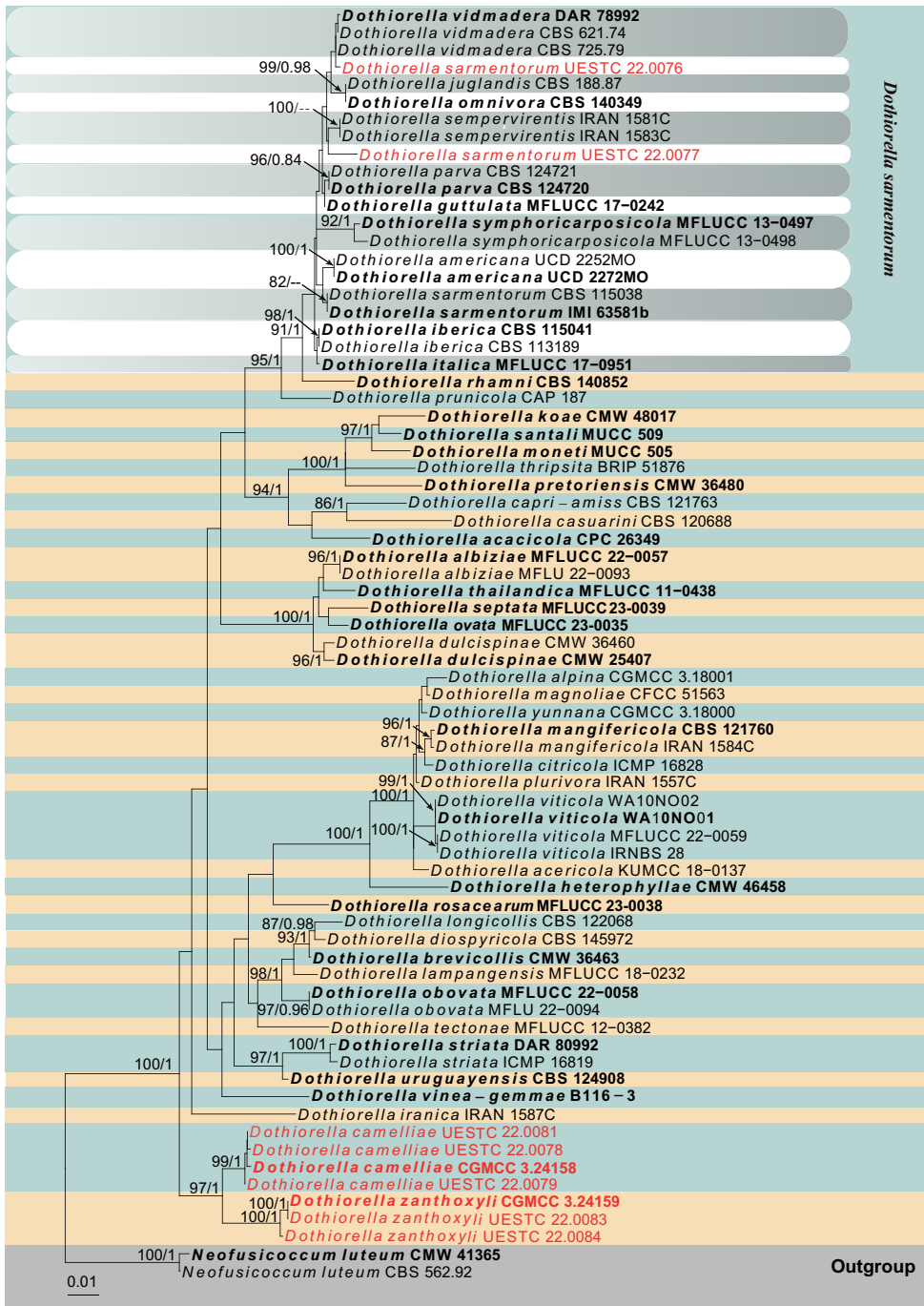
| Character                                                                     |             | Overview<br>phylogenetic tree         | <i>Botryosphaeria</i>                                                             | <i>Diplodia</i>                                                                           | <i>Dothiorella</i>                                                                   |
|-------------------------------------------------------------------------------|-------------|---------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Number of base pairs in each gene region (including the gaps after alignment) |             | ITS (603 bp), <i>tef1</i> (320 bp)    | ITS (555 bp), <i>tef1</i> (315 bp)                                                | ITS (537 bp), <i>tef1</i> (311 bp), <i>tub2</i> (381 bp)                                  | ITS (523 bp), <i>tef1</i> (294 bp), <i>tub2</i> (427 bp)                             |
| Number of isolates obtained in this study                                     |             | 17                                    | 12                                                                                | 11                                                                                        | 9                                                                                    |
| Number of taxa originated from GenBank                                        |             | 94                                    | 45                                                                                | 64                                                                                        | 73                                                                                   |
| Outgroup taxa                                                                 |             | <i>Lecanosticta acicola</i> (LNPV252) | <i>Barriopsis iraniana</i> (IRAN1448C) and <i>Barriopsis iraniana</i> (IRAN1449C) | <i>Dothiorella dulcispinae</i> (CMW 36460) and <i>Dothiorella dulcispinae</i> (CMW 36462) | <i>Neofusicoccum luteum</i> (CBS 562.92) and <i>Neofusicoccum luteum</i> (CMW 41365) |
| BI (model of each gene region)                                                | ITS         | GTR+I+G                               | SYM                                                                               | K80+I+G                                                                                   | HKY+I+G                                                                              |
|                                                                               | <i>tef1</i> | GTR+I+G                               | HKY+G                                                                             | GTR+G                                                                                     | GTR+G                                                                                |
|                                                                               | <i>tub2</i> | –                                     | –                                                                                 | GTR+G                                                                                     | GTR+I+G                                                                              |



**Figure 2.** Phylogram generated from RAxML analysis based on combined ITS and *tef1* sequence data of *Botryosphaeria* isolates. The tree was rooted to *Barriopsis iraniana* (IRAN1448C and IRAN1449C). The ML ( $\geq 75\%$ ) and BI ( $\geq 95\%$ ) bootstrap supports are given near the nodes, respectively. Isolates from this study are marked in red and ex-type strains are marked in bold.



**Figure 3.** Phylogram generated from RAxML analysis based on combined ITS, *tef1* and *tub2* sequence data of *Diplodia* isolates. The tree was rooted to *Dothiorella dulcispinae* (CMW 36460 and CMW 36462). The ML ( $\geq 75\%$ ) and BI ( $\geq 95\%$ ) bootstrap supports are given near the nodes, respectively. Isolates from this study are marked in red and ex-type strains are marked in bold.



**Figure 4.** Phylogram generated from RAxML analysis based on combined ITS, *tef1* and *tub2* sequence data of *Dothiorella* isolates. The tree was rooted to *Neofusicoccum luteum* (CBS 562.92 and CMW 41365). The ML ( $\geq 75\%$ ) and BI ( $\geq 95\%$ ) bootstrap supports are given near the nodes, respectively. Isolates from this study are marked in red and ex-type strains are marked in bold.

## Taxonomy

*Aplosporella ginkgonis* C.M. Tian, Z. Du & K.D. Hyde. *Mycosphere* 8(2): 1249 (2017).

MycoBank No: 552938

Fig. 5

**Description.** *Saprobic* on decaying branches of *Zanthoxylum bungeanum*. **Sexual morph:** Not observed. **Asexual morph:** Coelomycetous, *Conidiomata* 558–657 × 216–241 µm ( $\bar{x}$  = 235.5 × 228.5 µm, n = 10), immersed, partially erumpent when mature, multilocular, locules separated by pale brown cells of *textura angularis*. **Peridium** 65–106 µm wide, wall 6–10 cell-layers thick, outer layers composed of 3–4 layers of pale brown cells of *textura globulosa*, intermediate layers composed of dark brown cells of *textura angularis*, becoming pale brown towards the inner region. **Ostiole** 138–171 µm diam., central. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** 12–13 × 7.5–8 µm ( $\bar{x}$  = 12.5 × 8 µm, n = 20), holoblastic, hyaline, cylindrical to doliiform, smooth-walled. **Conidia** 17–20 × 6.5–7.5 µm ( $\bar{x}$  = 18.5 × 7 µm, n = 30), L/W ratio = 2.5, ellipsoidal to subcylindrical, with both ends rounded, initially hyaline, becoming dark brown, aseptate.

**Culture characteristics.** Colonies on PDA developing dense aerial mycelium with age, becoming white to gray-brown at the surface, and whitish to yellowish brown at the reverse, producing a brown pigment, with sinuate edges.

**Material examined.** CHINA, Sichuan Province, Yaan City, Hanyuan County, 29°16'51"N, 102°37'48"E, elevation 1,689 m, on dead branches of *Zanthoxylum bungeanum*, 30<sup>th</sup> October 2021, W.L. Li, HJ 511 (HUEST 22.0092), living culture UESTCC 22.0091.

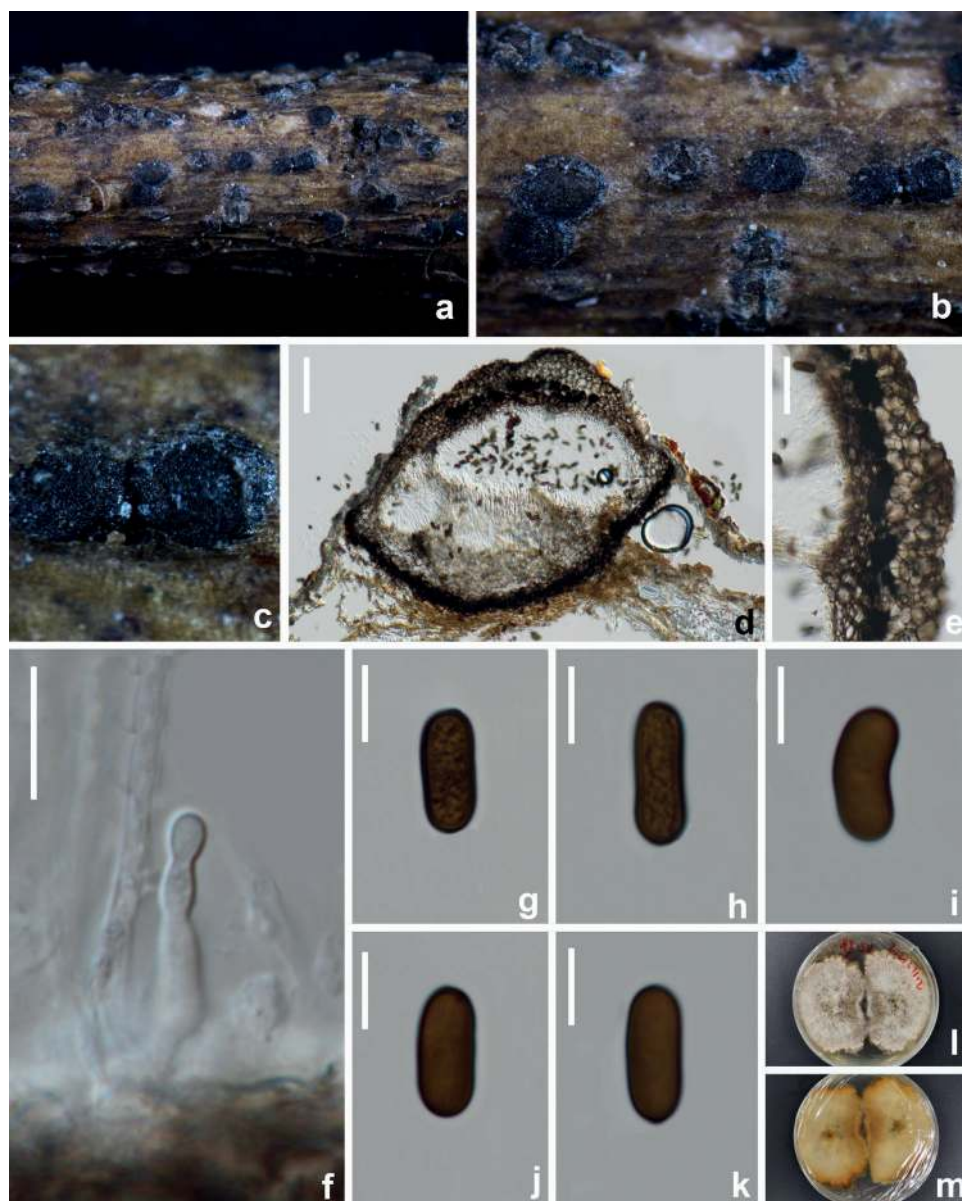
**Notes.** *Aplosporella ginkgonis* was introduced by Du et al. (2017) and isolated from diseased branches of *Ginkgo biloba* and *Morus alba* from Gansu Province in China. One isolate (UESTCC 22.0091) obtained in this study from *Zanthoxylum bungeanum* is morphologically similar to the original description of *Aplosporella ginkgonis*, and the sequences data are identical to the previous data (99%–100%). We, thus, identify the new collection as *Aplosporella ginkgonis* and this is the first report from *Zanthoxylum bungeanum*.

*Aplosporella prunicola* Damm & Crous *Fungal Diversity* 27: 39 (2007).

MycoBank No: 504373

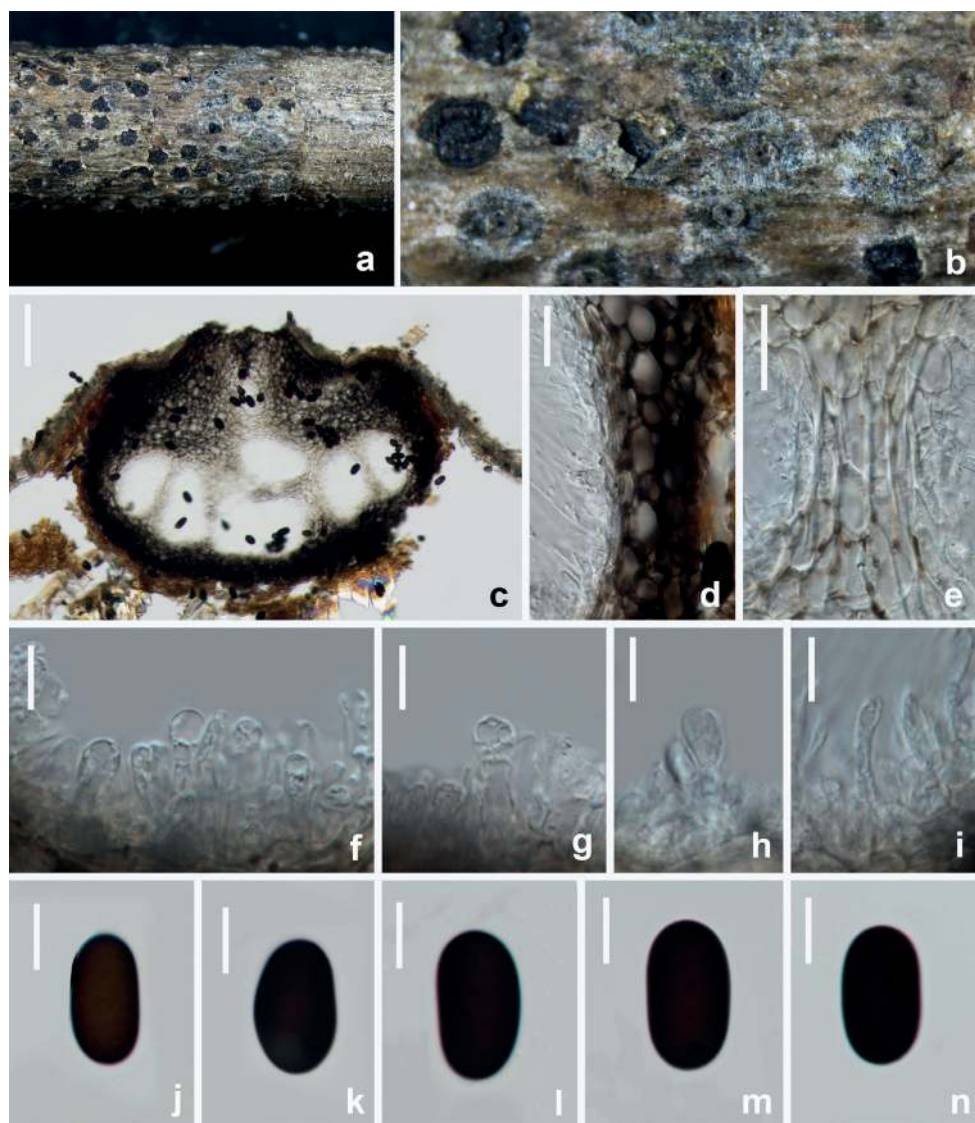
Fig. 6

**Description.** *Saprobic* on decaying branches of *Zanthoxylum bungeanum*. **Sexual morph:** Not observed. **Asexual morph:** Coelomycetous, *Conidiomata* 355–408 × 568.5–599 µm ( $\bar{x}$  = 381.5 × 584 µm, n = 10), immersed, partially erumpent when mature, multilocular, locules divided by pale brown cells of *textura angularis*.



**Figure 5.** *Aplosporella ginkgonis* (HUEST 22.0092, new host record) **a–c** appearance of conidiomata on natural substrate **d** vertical section of conidioma **e** section of peridium **f** conidiogenous cells and developing conidia **g–k** brown aseptate conidia **l** upper view of the colony on PDA after 14 d **m** reverse view of the colony on PDA after 14 d. Scale bars: 100  $\mu$ m (**d**); 40  $\mu$ m (**e**); 10  $\mu$ m (**f–k**).

**Peridium** 107–122  $\mu$ m wide, composed of 3–5 layers of pale brown cells of *textura globulosa*. **Ostiole** 70–88  $\mu$ m diam., central. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** 6.5–10  $\times$  2–3  $\mu$ m ( $\bar{x}$  = 8  $\times$  2.5  $\mu$ m,  $n$  = 20), holoblastic, hya-



**Figure 6.** *Aplosporella prunicola* (HUEST 22.0091, new host record) **a, b** appearance of conidiomata on natural substrate **c** vertical section of multiloculate conidioma **d, e** section of peridium **f–i** conidiogenous cells and developing conidia **j–n** brown aseptate conidia. Scale bars: 100  $\mu\text{m}$  (**c**); 20  $\mu\text{m}$  (**d, e**); 10  $\mu\text{m}$  (**f–n**).

line, cylindrical, smooth-walled. **Conidia** 20–23.5  $\times$  12–13.5  $\mu\text{m}$  ( $\bar{x}$  = 21.5  $\times$  13  $\mu\text{m}$ ,  $n$  = 30), L/W ratio = 1.6, ellipsoidal to subcylindrical, with both ends broadly rounded, initially hyaline, becoming dark brown, aseptate, smooth.

**Culture characteristics.** Colonies on PDA after 7 d, becoming pale olivaceous-gray to olivaceous-black at the surface, and olivaceous black at the reverse, with irregular edges.

**Material examined.** CHINA, Sichuan Province, Yaan City, Hanyuan County, 29°16'51"N, 102°37'48"E, elevation 1,689 m, on dead branches of *Zanthoxylum bungeanum*, 30<sup>th</sup> October 2021, W.L Li, HJ 509 (HUEST 22.0091), living culture UESTCC 22.0090.

**Notes.** Our isolate UESTCC 22.0090 morphologically lines up with the description of *Aplosporella prunicola* provided by Damm et al. (2007) in having immersed to erumpent, multilocular conidiomata and brown, smooth-walled, ovoid to oblong conidia. The strain UESTCC 22.0090 is phylogenetically and morphologically similar to *A. yalgorensis* and *A. prunicola*, however, *A. yalgorensis* can be distinguished from other *Aplosporella* species by its pitted conidial walls. Thus, the strain UESTCC 22.0090 was identified as *A. prunicola* based on current evidence. This is the first time *A. prunicola* is reported from *Zanthoxylum bungeanum* in China.

***Diplodia acerigena* L.W. Li & Jian K. Liu, sp. nov.**

Mycobank No: 847163

Figs 7, 8

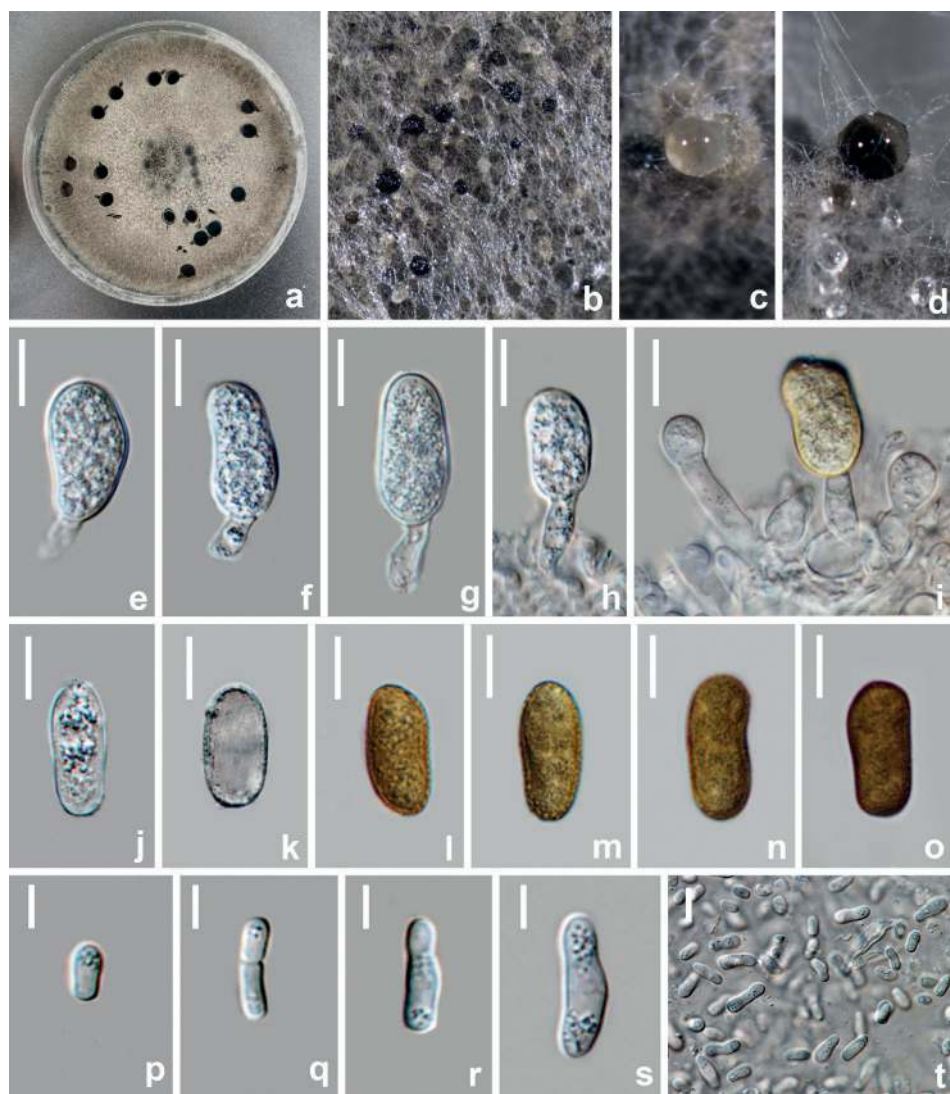
**Etymology.** The epithet “acerigena” refers to the host genus *Acer*, on which the holotype was collected.

**Holotype.** HKAS 125891.

**Description.** *Saprobic* on decaying branches of *Acer truncatum*. **Sexual morph:** *Ascomata* 304.5–321 × 217–260 ( $\bar{x}$  = 313 × 238.5  $\mu$ m,  $n$  = 20), more or less subglobose, solitary or gregarious, semi-immersed, medium brown to dark brown, unilocular, papillate, ostiolate. **Ostiole** 101–115  $\mu$ m diam., conical or circular, central, papillate, periphysate. **Peridium** 23–29  $\mu$ m wide, composed of 3–5 layers of dark brown cells of *textura angularis*. **Pseudoparaphyses** 3.5–5  $\mu$ m wide, hyaline, branched, septate. **Asci** 98–120 × 24–32.5  $\mu$ m ( $\bar{x}$  = 109 × 28  $\mu$ m,  $n$  = 30), (4–)8-spored, clavate, stipitate, irregularly bitunicate, apex rounded with an ocular chamber. **Ascospores** 24.5–31.5 × 13.5–16  $\mu$ m ( $\bar{x}$  = 28 × 14.5  $\mu$ m,  $n$  = 30), L/W ratio = 2, biserial, broadly fusiform to oval, widest in the middle, both ends obtuse, hyaline, moderately thick-walled, smooth, becoming brown and 2-septate when aged. **Asexual morph:** Coelomycetous, pycnidia produced on mycelium in PDA. **Conidiomata** stromatic, mostly solitary, gray to black, globose to subglobose. **Paraphyses** 2–3.5  $\mu$ m wide, hyaline, subcylindrical, branched, septate. **Conidiophores** absent. **Conidiogenous cells** 9–12 × 3.5–5  $\mu$ m ( $\bar{x}$  = 10.5 × 4.5  $\mu$ m,  $n$  = 20), holoblastic, hyaline, cylindrical. **Conidia** 21–24 × 10–11  $\mu$ m ( $\bar{x}$  = 22.5 × 10.5  $\mu$ m,  $n$  = 30), L/W ratio = 2, aseptate, thick-walled, wall externally smooth, roughened on the inner surface, initially hyaline becoming dark brown, obovoid to ellipsoid, both ends broadly rounded. **Spermatogenous cells** 7–9.5 × 2.5–3.5  $\mu$ m ( $\bar{x}$  = 8 × 3  $\mu$ m,  $n$  = 20), discrete or integrated, hyaline, smooth, cylindrical, holoblastic or proliferating via determinate phialides with periclinal thickening. **Spermatia** 7–11.5 × 3–4  $\mu$ m ( $\bar{x}$  = 9 × 3.5  $\mu$ m,  $n$  = 30), hyaline, smooth, aseptate, rod-shaped with rounded ends.



**Figure 7.** The sexual morph of *Diplodia acerigena* (HKAS 125891, holotype) **a, b** appearance of ascomata on natural substrate **c** vertical section of ascoma **d** ostiole **e** section of peridium **f–h** asci with hyaline ascospores **i** asci with brown 2-septate ascospores **j, k** hyaline immature aseptate ascospores **l–n** mature brown 2-septate ascospores **o** germinated ascospore **p** upper view of the colony on PDA after 14 d **q** reverse view of the colony on PDA after 14 d. Scale bars: 100  $\mu\text{m}$  (**c**); 10  $\mu\text{m}$  (**d, e, j–o**); 20  $\mu\text{m}$  (**f–i**).



**Figure 8.** The asexual morph of *Diplodia acerigena* (HKAS 125891, holotype) **a–d** appearance of conidiomata on PDA **e–i** conidiogenous cells and developing conidia **j, k** hyaline immature conidia **l–o** mature brown aseptate conidia **p–t** Spermatogenous cells and Spermatia. Scale bars: 10  $\mu$ m (**e–o, t**); 5  $\mu$ m (**p–s**).

**Culture characteristics.** Ascospores germinating on PDA within 12 h. Colonies growing on PDA, reaching a diam. of 4 cm after five days at 25 °C, effuse, velvety, with entire to slightly undulate edge. Surface initially white and later turning dark olivaceous from the surrounding of the colony and dark gray in reverse.

**Material examined.** CHINA, Sichuan Province, Chengdu City, Pidu District, 30°19'57"N, 103°59'47"E, elevation 442 m, on dead branches of *Acer truncatum* (Anacardiaceae), 19<sup>th</sup> March 2021, W.L Li, YBF 96 (HKAS 125891, holotype), ex-

type living culture UESTCC 22.0073 = CGMCC 3.24157; *ibid.*, YBF103 (HUEST 22.0075, paratype), living culture UESTCC 22.0074. Additional sequences: LSU: OQ164827 (CGMCC 3.24157), OQ164828 (UESTCC 22.0074).

**Notes.** Three isolates of *Diplodia acerigena* clustered closer to *Di. pseudoseriata* (CBS 124906) with high bootstrap support (ML/Bi 100%/1). The asexual morph of *Diplodia pseudoseriata* was introduced by Pérez et al. (2010), collected and isolated from the *Blepharocalyx salicifolius* in Uruguay and its sexual morph has not been reported. The asexual morph of *Diplodia acerigena* differs from *Di. pseudoseriata* in having conidia which become 1-septate when aged. *Diplodia acerigena* shares similar sexual morph characters as of other *Diplodia* species by having immersed to semi-immersed pseudothecia, clavate asci, broadly fusiform to ovoid and hyaline ascospores. However, conidia of *Diplodia acerigena* become brown and septate when aged, which is rarely observed in any other sexual morph species of this genus.

***Diplodia mutila* (Fr.) Mont., Ann. Sci. nat., sér. 2, 1: 302. 1834.**

MycoBank No: 201741

Fig. 9

*Sphaeria mutila* Fr., Syst. Mycol. (Lundae) 2: 424. 1823. Basionym.

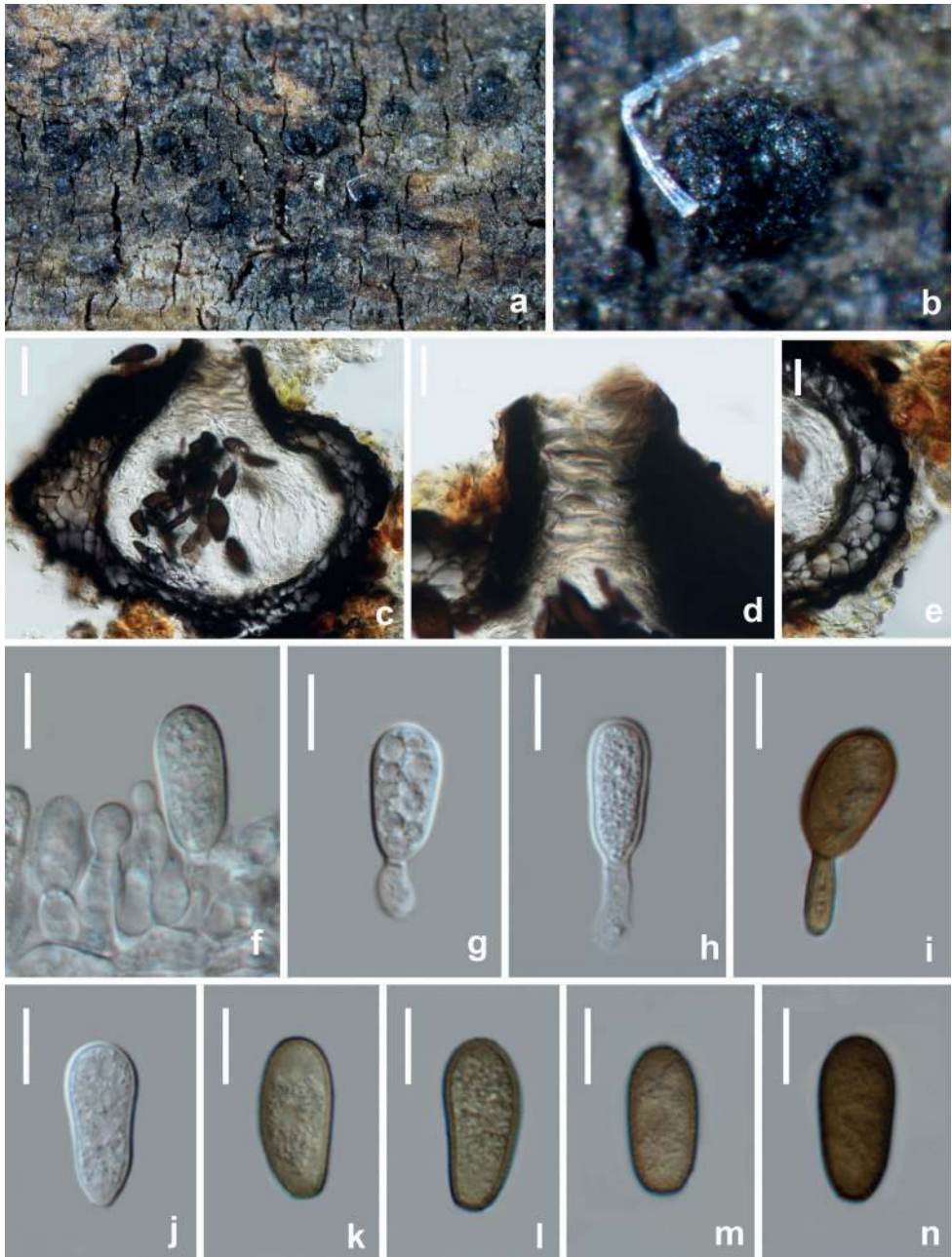
≡ *Physalospora mutila* (Fr.) N.E. Stevens, Mycologia 28: 333. 1936.

= *Botryosphaeria stevensii* Shoemaker, Canad. J. Bot. 42: 1299. 1964.

**Description.** *Saprobic* on decaying branches of *Camellia oleifera*. **Sexual morph:** Not observed. **Asexual morph:** Coelomycetous, **Conidiomata** 330–394 × 215–230 µm ( $\bar{x}$  = 362 × 223 µm, n = 10), immersed, erumpent, gregarious, dark brown to black, subglobose, unilocular. **Ostiole** 48.5–67 µm diam., central. **Peridium** 29–38 µm wide, thick-walled, outer and inner layers composed of 1–2 layers dark brown **textura angularis**, intermediate layers composed of 3–5 layers of hyaline cells of **textura angularis**. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** 8.5–12 × 3–5 µm ( $\bar{x}$  = 10 × 4 µm, n = 20), cylindrical, thin-walled, hyaline, holoblastic, indeterminate, proliferating at the same level to produce periclinal thickenings, or proliferating percurrently giving rise to 2–3 indistinct annellations. **Conidia** 19–21 × 9.5–11 µm ( $\bar{x}$  = 20 × 10.5 µm, n = 30), L/W ratio = 2, oblong, with broadly rounded apex and truncate base, thick-walled, wall externally smooth, roughened on the inner surface, hyaline, aseptate, becoming dark brown when aged.

**Culture characteristics.** Colonies on PDA initially olivaceous buff in the center of the colony and white at the edge, becoming olivaceous within 7 d on the surface, with smooth edge.

**Materials examined.** CHINA, Sichuan Province, Jiangyou City, Shuanghe County, 31°54'10"N, 104°55'57"E, elevation 657 m, on dead branches of *Camellia oleifera*,



**Figure 9.** *Diplodia mutila* (HUEST 22.0069, new host record) **a, b** appearance of conidiomata on natural substrate **c** vertical section of conidioma **d** ostiole **e** section of peridium **f–i** conidiogenous cells and developing conidia **j** hyaline immature conidium **k–n** mature brown conidia. Scale bars: 40 µm (**c**); 20 µm (**d, e**); 10 µm (**f–n**).

11<sup>th</sup> July 2021, W.L Li, 286 (HUEST 22.0069), living culture UESTCC 22.0068; *ibid.*, 289 (HUEST 22.0068), living culture UESTCC 22.0067; *ibid.*, Guangyuan city, Qingchuan County, 32°40'38"N, 105°28'57"E, elevation 634 m, on dead branches of *Olea europaea*, 20<sup>th</sup> April 2021, W.L Li, 188 (HUEST 22.0065), living culture UESTCC 22.0064; *ibid.*, 257 (HUEST 22.0070), living culture UESTCC 22.0069; *ibid.*, on dead branches of *Vernicia fordii*, 20<sup>th</sup> April 2021, W.L Li, 238 (HUEST 22.0066), living culture UESTCC 22.0065; *ibid.*, Chengdu City, Pidu District, 30°49'27"N, 103°47'42"E, elevation 442 m, on dead branches of *Pistacia chinensis*, 5<sup>th</sup> March 2021, W.L Li, A61 (HUEST 22.0064), living culture UESTCC 22.0063. Additional sequences: LSU: OQ164832 (UESTCC 22.0063), OQ164830 (UESTCC 22.0064), OQ164831 (UESTCC 22.0065).

**Notes.** The phylogenetic tree show that six strains isolated from *Camellia oleifera*, *Olea europaea* and *Vernicia fordii* nested with *Diplodia mutila* (CBS 112553) with a moderate bootstrap support (ML/BI 86%/1). *Diplodia mutila*, the type of the genus, is a well-known and most commonly reported species. It has been recorded mainly from woody substrates, and it is known from more than 50 hosts (Batista et al. 2021). Morphologically, one of the isolates obtained in this study UESTCC 22.0068 shares similar conidia shape and size with *Di. mutila*, but hardly observed the mature conidia with septa. We identify these taxa as *Di. mutila* based on morphology and phylogeny evidences. This is the first report of *Di. mutila*, isolated from *Camellia oleifera*, *Olea europaea* and *Vernicia fordii*.

### ***Diplodia pistaciicola* L.W. Li & Jian K. Liu, sp. nov.**

MycoBank No: 847166

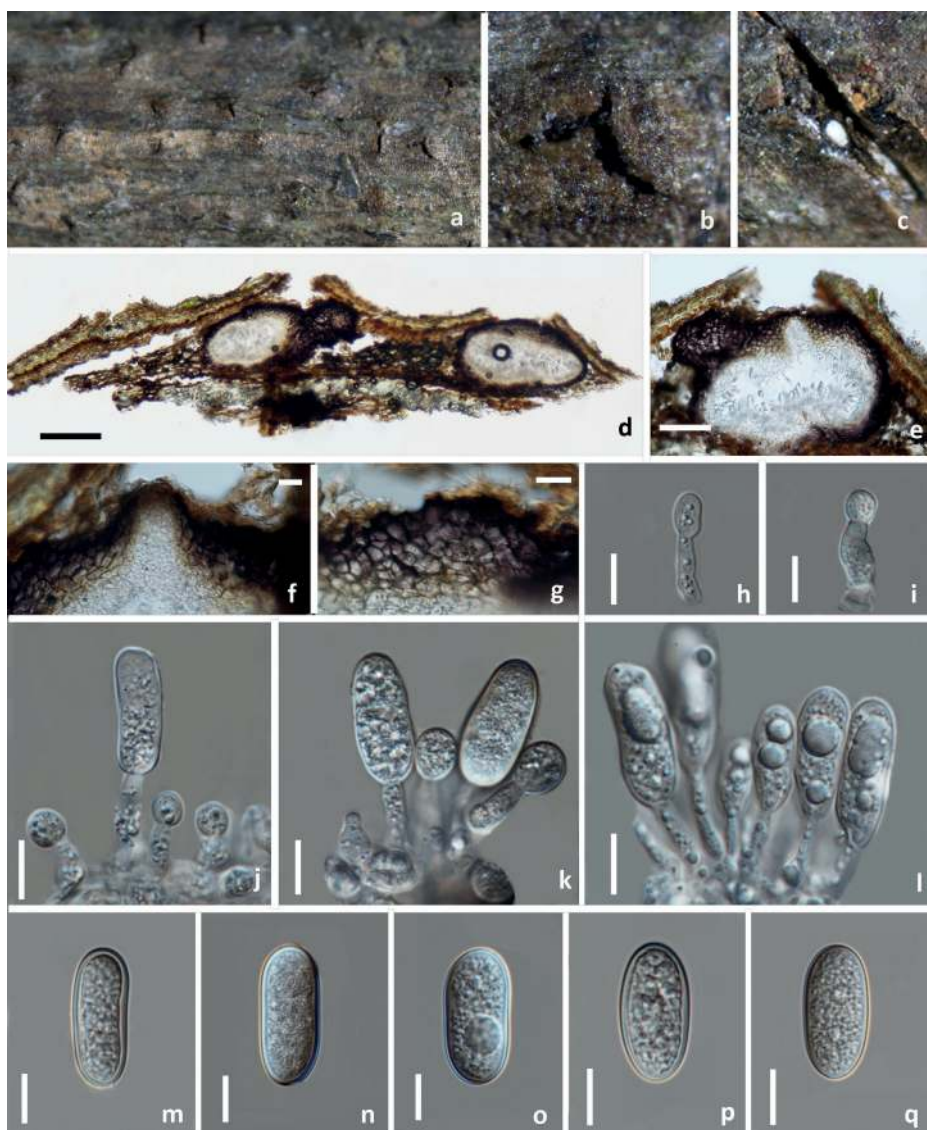
Fig. 10

**Etymology.** The epithet “*pistaciicola*” refers to the host genus *Pistacia*, on which the holotype was collected.

**Holotype.** HKAS 125890.

**Description.** *Saprobic* on decaying branches of *Pistacia chinensis*. **Sexual morph:** Not observed. **Asexual morph:** Coelomycetous, ***Conidiomata*** 353–441 × 274.5–316 µm ( $\bar{x}$  = 397 × 295 µm, n = 10), immersed, forming split-like opening on the host, solitary or gregarious, globose to subglobose, dark brown to black, unilocular, papillate, ostiolate. ***Ostiole*** 38–49.5 µm diam., conical or circular, centrally located. ***Peridium*** 42–60 µm wide, composed of thick walled, dark brown to hyaline cells of ***textura angularis***. ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** 10–14 × 3–4 µm ( $\bar{x}$  = 12 × 3.5 µm, n = 20), holoblastic, discrete, cylindrical, hyaline, smooth, indeterminate, arising from the inner cavity of the conidiomata. ***Conidia*** 24.5–27 × 11–13 µm ( $\bar{x}$  = 25.5 × 12 µm, n = 30), L/W ratio = 2.2, ellipsoid to obovoid, aseptate, hyaline, thick-walled, guttulate.

**Culture characteristics.** Conidia germinate on PDA within 12 h. Colonies growing on PDA, reaching a diameter of 4 cm after five days at 25 °C, effuse, velvety, with



**Figure 10.** *Diplodia pistaciicola* (HKAS 125890, holotype) **a–c** appearance of conidiomata on natural substrate **d, e** vertical section of conidiomata/conidioma **f** ostiole **g** section of peridium **h–l** conidiogenous cells and developing conidia **m–q** hyaline aseptate conidia. Scale bars: 200 µm (**d**); 50 µm (**e**); 20 µm (**f, g**); 10 µm (**h–q**).

entire to slightly undulate edge. The early stage of the colony is white, later turning dark olivaceous and dark gray in reverse.

**Material examined.** CHINA, Sichuan Province, Chengdu City, Piddu District, 30°49'27"N, 103°47'42"E, elevation 442 m, on dead branches of *Pistacia chinensis* (Anacardiaceae), 5<sup>th</sup> March 2021, W.L Li, 049 (HKAS 125890, holotype), ex-type

living culture UESTCC 22.0070 = CGMCC 3.24156; *ibid.*, 049B (HUEST 22.0072 isotype), ex-isotype living culture UESTCC 22.0071. Additional sequence: LSU: OQ164833 (CGMCC 3.24156).

**Notes.** Phylogenetic analyses showed that two strains of *Diplodia pistaciicola* isolated from *Pistacia chinensis* are distinct but closely related to *Di. agrifolia* (CBS 124.30). The comparison of ITS, *tef1* and *tub2* of these two species indicate 5 bp (502), 3bp (224), 9 bp (425) differences, respectively. Morphologically, *Di. agrifolia* differs from *Di. pistaciicola* in producing two to three times larger ascomata than that of *Di. pistaciicola* (721–836 vs. 274.5–316  $\mu\text{m}$ ) and possessing smaller conidia (27–36.5  $\times$  14.5–17.8  $\mu\text{m}$  vs. 24.5–27  $\times$  11–13  $\mu\text{m}$ ). In addition, conidia of *Di. pistaciicola* are hyaline, aseptate, rarely becoming pale brown and uniseptate with age, whereas conidia of *Di. agrifolia* are mostly dark brown and uniseptate before discharge from pycnidia.

***Diplodia seriata* De Not., Micr. Ital. Dec. 4: 6. (1942).**

MycoBank No: 180468

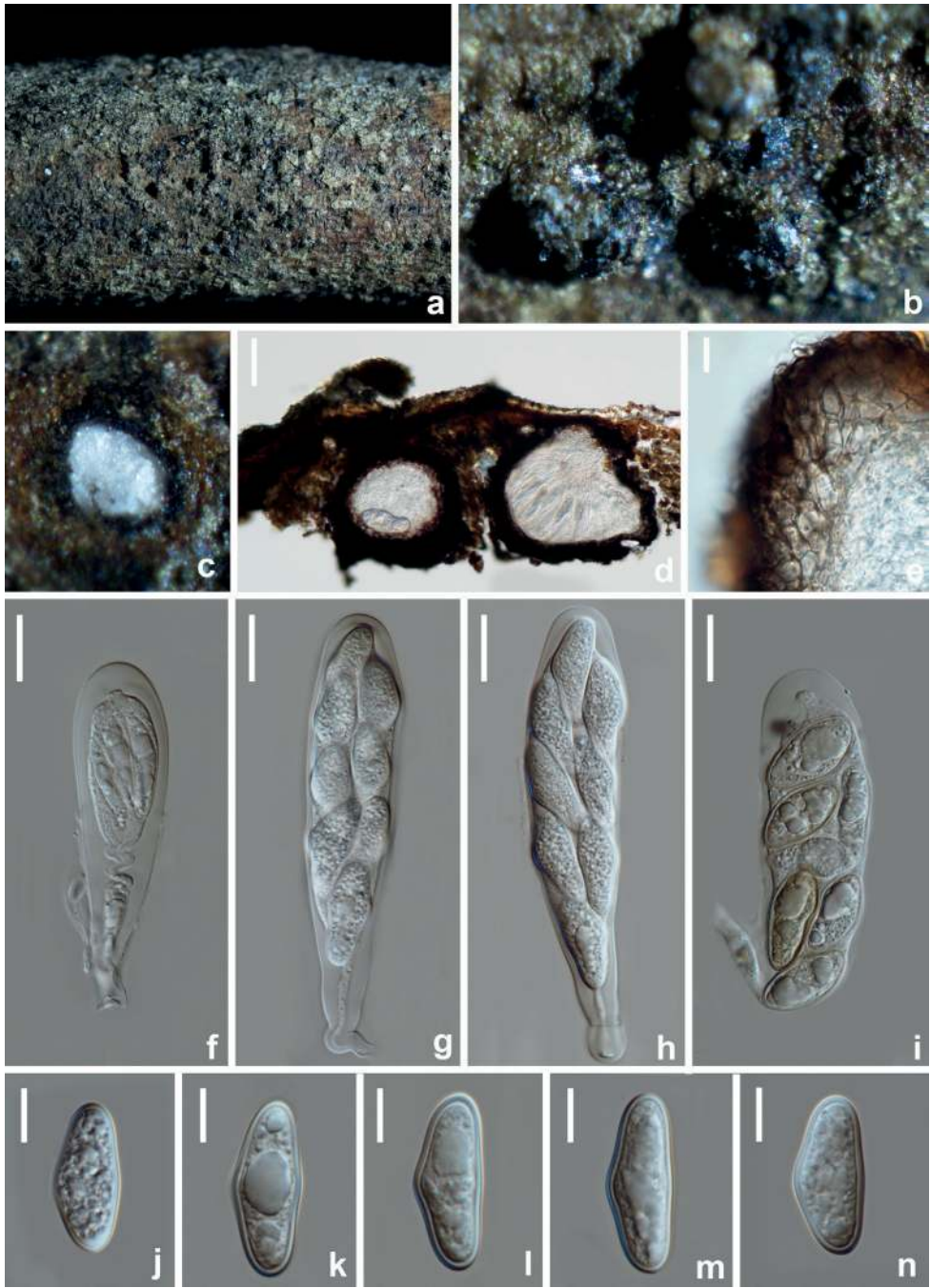
Fig. 11

**Description.** *Saprobic* on decaying branches of *Camellia oleifera*. **Sexual morph:** **Ascomata** 301–343  $\times$  293–340 ( $\bar{x}$  = 322  $\times$  316  $\mu\text{m}$ ,  $n$  = 10), more or less subglobose, solitary or gregarious, semi-immersed, medium brown to dark brown, unilocular, papillate, ostiolate. **Ostiole** 72–78  $\mu\text{m}$  diam., conical or circular, central, papillate, periphysate. **Peridium** 33–44  $\mu\text{m}$  wide, composed of dark brown, 4–6 layers of **textura angularis**. **Pseudoparaphyses** 2–2.5  $\mu\text{m}$  wide, hyaline, branched, septate. **Asci** 112–141  $\times$  27.5–30  $\mu\text{m}$  ( $\bar{x}$  = 126  $\times$  28.5  $\mu\text{m}$ ,  $n$  = 30), clavate, stipitate, bitunicate, containing eight, biserial ascospores. **Ascospores** 31.5–32.5  $\times$  12–13.5  $\mu\text{m}$  ( $\bar{x}$  = 32  $\times$  13  $\mu\text{m}$ ,  $n$  = 30), L/W ratio = 2.5, broadly fusiform to oval, widest in the middle, both ends obtuse, hyaline, moderately thick-walled, smooth, becoming brown when aged. **Asexual morph:** Not observed.

**Culture characteristics.** Ascospores germinate on PDA within 12 h. Colonies growing on PDA, reaching a diameter of 4 cm after five days at 25 °C, effuse, velvety, with entire to slightly undulate edge.

**Material examined.** CHINA, Sichuan Province, Jiangyou City, shuanghe County, 31°54'10"N, 104°55'57"E, elevation 656 m, on dead branches of *Camellia oleifera*, 10<sup>th</sup> June 2021, W.L Li, 288 (HUEST 22.0073), living culture UESTCC 22.0072.

**Notes.** The morphology of the taxa isolated from decaying woody oil plants is similar to *Diplodia seriata*. In the multi-gene phylogenetic analysis, our new collection clustered with the ex-type strain of *Di. seriata* (CBS 112555) with strong bootstrap support. *Diplodia seriata* has been isolated from a wide range of hosts (121 species) and has a worldwide distribution (reported in 46 countries) (Batista et al. 2021). This is the first report of *Di. seriata* isolated from *Camellia oleifera*.



**Figure 11.** *Diplodia seriata* (HUEST 22.0073, new host record) **a–c** appearance of ascomata on natural substrate **d** vertical section of ascomata **e** section of peridium **f–i** asci **j–n** ascospores. Scale bars: 100  $\mu\text{m}$  (**d**); 10  $\mu\text{m}$  (**e**, **j–n**); 20  $\mu\text{m}$  (**f–i**).

***Dothiorella camelliae* L.W. Li & Jian K. Liu, sp. nov.**

MycoBank No: 847167

Fig. 12

**Etymology.** The epithet “*camelliae*” refers to the host genus *Camellia*, on which the holotype was collected.

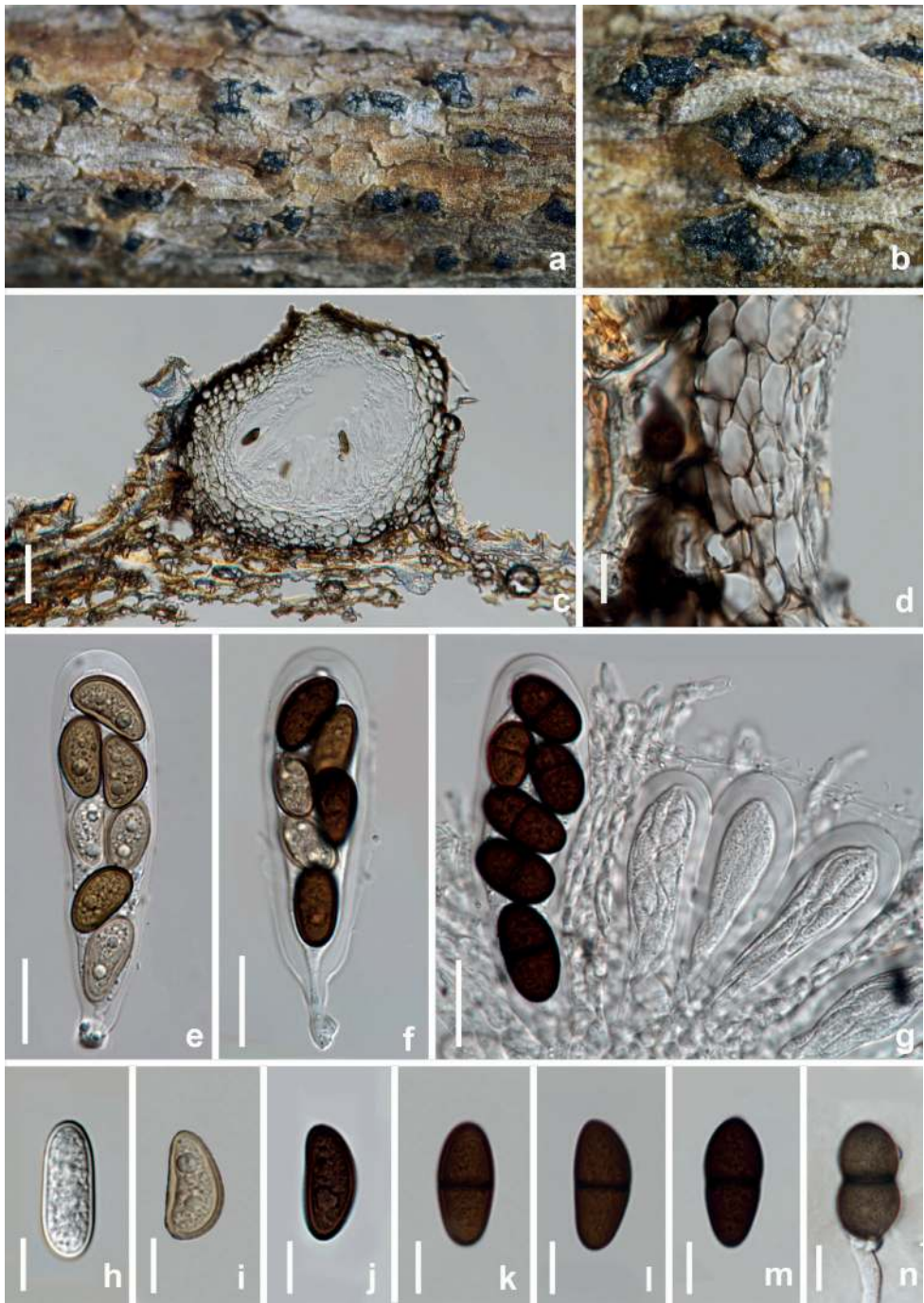
**Holotype.** HKAS 125892.

**Description.** *Saprobic* on decaying branches of *Camellia oleifera*. **Sexual morph:** *Ascomata* 199–222 × 237–269 µm ( $\bar{x}$  = 210.5 × 253 µm, n = 10), submerged in the substrate, partly erumpent at maturity, solitary or gregarious, dark brown to black, subglobose, multilocular or unilocular. *Ostiole* 17–37 µm diam., central. *Peridium* 35–43 µm wide, thick-walled, outer layers composed of 1–2 layers dark brown cells of *textura angularis*, becoming hyaline towards the inner region. *Pseudoparaphyses* 3–4 µm wide, hyaline, frequently aseptate. *Asci* 80–96 × 22–25 µm ( $\bar{x}$  = 88 × 23.5 µm, n = 30), stipitate, clavate, thick-walled, bitunicate, (6–)8-spored, irregularly biseriate. *Ascospores* 21–25 × 9.5–12 µm ( $\bar{x}$  = 23 × 10.5 µm, n = 30), L/W ratio = 2, oblong, ovate to sub-clavate, (0–)1-septate, slightly constricted at the septum, hyaline to dark brown, moderately thick-walled, straight or inequilateral, basal cell tapering towards the acute end. **Asexual morph:** Not observed.

**Culture characteristics.** Ascospores germinate on PDA within 12 h. Colonies growing on PDA, reaching a diameter of 4 cm after five days at 25 °C, effuse, velvety, with entire to slightly undulate edge. Surface initially white and later turning dark olivaceous from the surrounding of the colony and dark gray in reverse.

**Materials examined.** CHINA, Sichuan Province, Leshan City, Wutongqiao District, 29°22'28"N, 103°45'49"E, elevation 383 m, on dead branches of *Camellia oleifera* (Theaceae), 23<sup>th</sup> July 2021, Z.P Liu, 351 (HKAS 125892, holotype), ex-type living culture UESTCC 22.0081 = CGMCC 3.24158; *ibid.*, 347 (HUEST 22.0081), living culture UESTCC 22.0080; *ibid.*, Shizhong District, 29°42'13"N, 103°52'25"E, elevation 356 m, on dead branches of *Paeonia suffruticosa*, 23<sup>th</sup> July 2021, W.L Li, A240 (HUEST 22.0080), living culture UESTCC 22.0079; *ibid.*, A234 (HUEST 22.0079), living culture UESTCC 22.0078. Additional sequences: LSU: OQ164834 (CGMCC 3.24158), OQ164835 (UESTCC 22.0079), OQ164836 (UESTCC 22.0078).

**Notes.** Four strains isolated from *Vernicia fordii* and *Camellia oleifera* occupy a basal position in the *Dothiorella* phylogenetic tree by forming a well-supported sub-clade sister to *Do. zanthoxyli* (ML/BI 97%/1, Fig. 4). The BLASTn searches of the ITS sequence of *Dothiorella zanthoxyli* resulted in 97% matches with *Neofusicoccum viti-fusiforme* BRIP64010, the *tef1* showed 91.23% matches with *Do. symphoricarposicola* BL158, and the *tub2* BLASTn results indicated 96.53% similarity with *Do. uruguayensis* CBS 124908 and *Do. viticola* B116-3. *Dothiorella camelliae* can be distinguished from *Do. zanthoxyli* in the size of ascomata, ascus and L/W ratio of ascospores (Table 3). *Dothiorella camelliae* resembles the sexual morph of *Do. sarmentorum* in producing immersed to sub-immersed ascomata, clavate asci and ovate to sub-clavate, hyaline to



**Figure 12.** *Dothiorella camelliae* (HKAS 125892, holotype) **a, b** appearance of ascomata on natural substrate **c** vertical section of ascoma **d** section of peridium **e–g** asci **h–m** ascospores **n** germinated ascospore. Scale bars: 50  $\mu\text{m}$  (**c**); 10  $\mu\text{m}$  (**d, h–n**); 20  $\mu\text{m}$  (**e–g**).

**Table 3.** A morphological comparison of the sexual morph of three *Dothiorella* species.

| Taxa                           | Ascomata (µm)     | Asci (µm)         | Peridium (µm) | Ascospores            |                       |           |
|--------------------------------|-------------------|-------------------|---------------|-----------------------|-----------------------|-----------|
|                                |                   |                   |               | Size(µm)              | Color                 | L/W ratio |
| <i>Dothiorella camelliae</i>   | 199–222 × 237–269 | 80–96 × 22–25     | 35–43         | 21–25 × 9.5–12        | Hyaline to dark brown | 2         |
| <i>Dothiorella sarmentorum</i> | 350–400           | 140–210 × 17–24   | 50–75         | 24.5–25.5 × 11.5–12.5 | Dark brown            | 2.4       |
| <i>Dothiorella zanthoxyli</i>  | 258–280 × 170–174 | 63.5–77 × 20–24.5 | 35–40         | 22.5–25 × 9.5–11      | Hyaline to dark brown | 2.6       |

brown conidia with (0–)1-septate. However, *Do. sarmentorum* morphologically can be distinguished from *Do. camelliae* in having larger ascomata (350–400 µm vs. 237–269 µm), thicker peridium (50–75 µm vs. 35–43 µm), and longer asci (140–210 µm vs. 80–96 µm) (Table 3). Phylogenetically, these two species reside in two distinct clades.

*Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, J. Luque & A. Alves, *Mycologia* 97: 522. (2005).

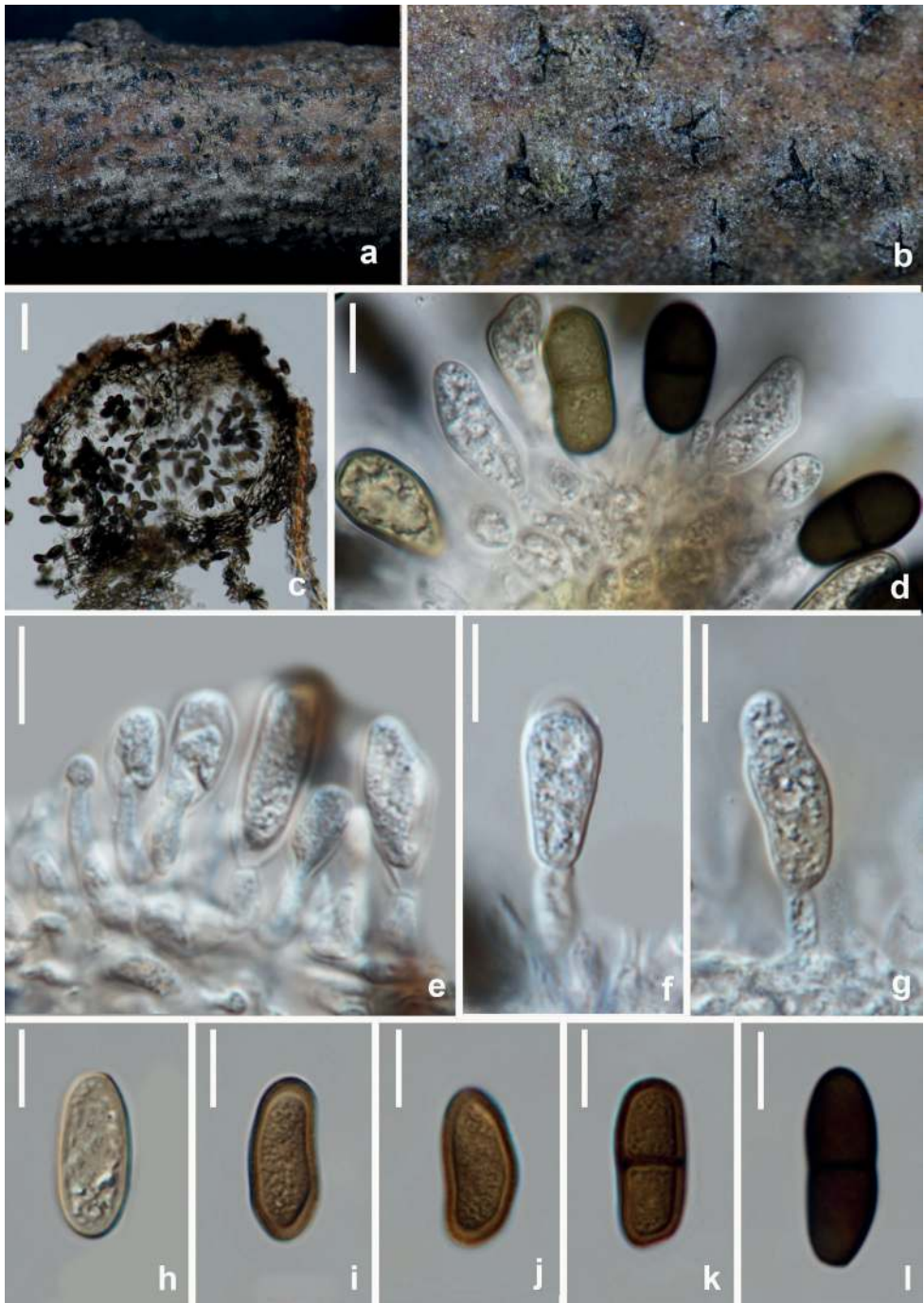
MycoBank No: 501403

Fig. 13

*Sphaeria sarmentorum* Fr., K. svenska Vetensk-Acad. Handl. 39: 107. 1818. Basionym.  
≡ *Diplodia sarmentorum* (Fr.) Fr., Summ. veg. Scand. (Stockholm) 2: 417. 1849.  
= *Diplodia pruni* Fuckel, Jahrb. Nassauischen Vereins Naturk., 23–24: 169. 1870 [1869].  
= *Botryosphaeria sarmentorum* A.J.L. Phillips, J. Luque & A. Alves, *Mycologia* 97: 522. 2005.

**Description.** *Saprobic* on decaying branches of *Pistacia chinensis*. **Sexual morph:** Not observed. **Asexual morph:** **Conidiomata** 278–338 × 240–280 µm ( $\bar{x}$  = 308 × 260 µm, n = 10), immersed, erumpent, forming split-like opening on the host, gregarious, globose to subglobose, dark brown to black, unilocular or multilocular, papillate, ostiole. **Ostiole** 52–57 µm diam., conical or circular, centrally located. **Peridium** 28.5–44 µm, comprising 5–8 layers of thick-walled, dark brown to hyaline cells arranged in a **textura angularis**. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** 2.5–3.5 × 6–9 µm ( $\bar{x}$  = 3 × 7.5 µm, n = 20), holoblastic, discrete, cylindrical, hyaline, smooth, indeterminate, proliferating at the same level giving rise to periclinal thickenings, or rarely proliferating percurrently to form one or two close, indistinct annellations. **Conidia** 21.5–24 × 9–10 µm ( $\bar{x}$  = 22.5 × 9.5 µm, n = 30), L/W ratio = 2.4, ellipsoid to obovoid, with rounded ends, initially hyaline and aseptate becoming pigmented brown and 1-septate often while still attached to conidiogenous cell, brown walled, slightly constricted at the septum.

**Culture characteristics.** Conidia germinate on PDA within 12 h. Colonies growing on PDA, reaching a diameter of 4 cm after three days at 25 °C, effuse, velvety, with



**Figure 13.** *Dothiorella sarmentorum* (HUEST 22.0077, new host record) **a,b** appearance of conidiomata on natural substrate **c** vertical section of conidioma **d–g** conidiogenous cells and developing conidia **h–i** brown conidia. Scale bars: 50  $\mu\text{m}$  (**c**); 10  $\mu\text{m}$  (**d–i**).

entire to slightly undulate edge. Surface initially white and later turning dark olivaceous from the surrounding of the colony and dark gray in reverse.

**Materials examined.** CHINA, Sichuan Province, Chengdu City, Pidu District, 30°19'57"N, 103°59'47"E, elevation 442 m, on dead branches of *Pistacia chinensis*, 19<sup>th</sup> March 2021, W.L Li, 072 (HUEST 22.0077), living culture UESTCC 22.0076; *ibid.*, Guangyuan City, Qingchuan County, 32°40'38"N, 105°28'57"E, elevation 638 m, 20<sup>th</sup> April 2021, W.L Li, A189 (HUEST 22.0078), living culture UESTCC 22.0077. Additional sequences: LSU: OQ164837 (UESTCC 22.0076), OQ164838 (UESTCC 22.0077).

**Notes.** *Dothiorella sarmentorum* was introduced by Phillips et al (2005) with both asexual and sexual morphs. Recently, nine *Dothiorella* species (*Do. californica*, *Do. iberica*, *Do. italica*, *Do. guttulata*, *Do. omnivora*, *Do. parva*, *Do. sempervirentis*, *Do. symphoricarpicola*, *Do. vidmadera*) were synonymized under *Do. Sarmentorum* by Zhang et al. (2021) based on phylogenetic analyses. Two isolates obtained in the present study clustered with the group of *Do. sarmentorum* taxa in the phylogenetic analyses (Fig. 4).

***Dothiorella zanthoxyli* L.W. Li & Jian K. Liu, sp. nov.**

MycoBank No: 847168

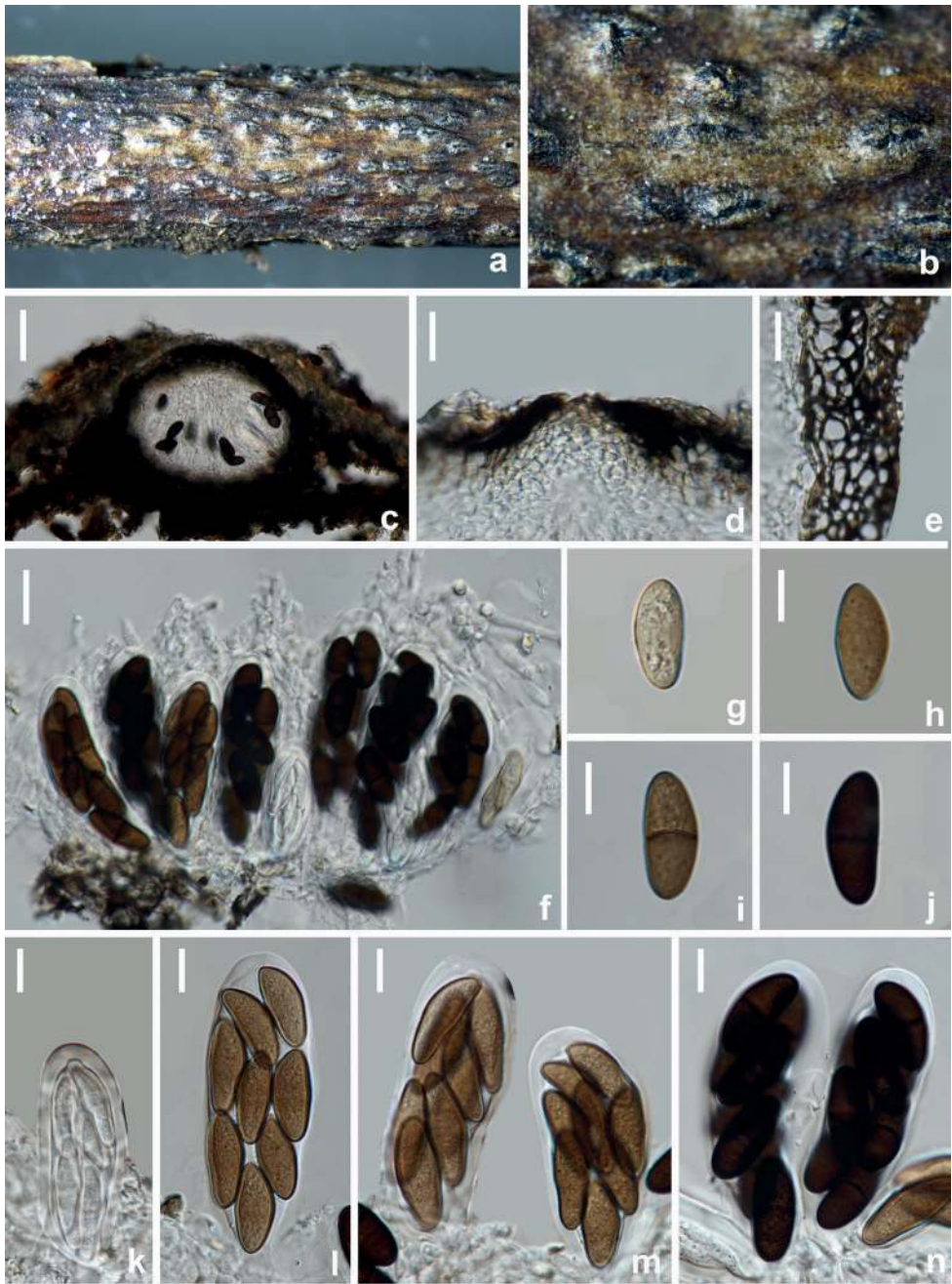
Fig. 14

**Etymology.** The epithet “*zanthoxyli*” refers to the host genus *Zanthoxylum*, on which the holotype was collected.

**Holotype.** HKAS 125893.

**Description.** *Saprobic* on decaying branches of *Zanthoxylum bungeanum*. **Sexual morph: Ascomata** 258–280 × 170–174 µm ( $\bar{x}$  = 269 × 172 µm, n = 10), submerged in the substrate, partly erumpent at maturity, solitary or gregarious, dark brown to black, subglobose, unilocular. **Ostiole** 42–44 µm diam., central. **Peridium** 35–40 µm wide, thick-walled, outer layers composed of 3–5 layers dark brown cells of *textura angularis*, becoming hyaline towards the inner region. **Pseudoparaphyses** 3–4.5 µm wide, hyaline, frequently aseptate. **Asci** 63.5–77 × 20–24.5 µm ( $\bar{x}$  = 70 × 22.5 µm, n = 30), short stipe, clavate, thick-walled, bitunicate, 8-spored, irregularly biseriate. **Ascospores** 22.5–25 × 9.5–11 µm ( $\bar{x}$  = 24 × 10 µm, n = 30), L/W ratio = 2.6, oblong, ovate to sub-clavate, (0–)1-septate, slightly constricted at the septum, hyaline to dark brown, moderately thick-walled, straight or inequilateral, basal cell tapering towards the acute end. **Asexual morph:** Not observed.

**Culture characteristics.** Ascospores germinate on PDA within 12 h. Colonies growing on PDA, reaching a diameter of 4 cm after five days at 25 °C, effuse, velvety, with entire to slightly undulate edge. Surface initially white and later turning dark olivaceous from the surrounding of the colony. Dark gray in reverse.



**Figure 14.** *Dothiorella zanthoxyli* (HKAS 125893, holotype) **a, b** appearance of ascomata on natural substrate **c** vertical section of ascoma **d** ostiole **e** section of peridium **f, k–n** asci **g–j** brown ascospores. Scale bars: 50  $\mu\text{m}$  (**c**); 20  $\mu\text{m}$  (**d–f**); 10  $\mu\text{m}$  (**g–n**).

**Materials examined.** CHINA, Sichuan Province, Yanan City, Hanyuan County, 29°16'51"N, 102°37'48"E, elevation 1,689 m, on dead branches of *Zanthoxylum bungeanum* (Rutaceae), 30<sup>th</sup> October 2021, W.L Li, 504 (HKAS 125893, holotype), ex-type living culture UESTCC 22.0082 = CGMCC 3.24159; *ibid.*, 506 (HUEST 22.0084), living culture UESTCC 22.0083; *ibid.*, 507 (HUEST 22.0085), living culture UESTCC 22.0084. Additional sequences: LSU: OQ164839 (CGMCC 3.24159), OQ164840 (UESTCC 22.0083), OQ164841 (UESTCC 22.0084).

**Notes.** Three strains of *Dothiorella zanthoxyli* isolated from *Zanthoxylum bungeanum* correspond well with sexual morph of *Dothiorella* described by Phillips et al. (2013), but morphologically differ from other species (*Do. camelliae*, *Do. iberica* and *Do. sarmentorum*) in the size of ascomata and asci (Table 3). A comparison of ITS and *tef1* nucleotides shows that *Do. zanthoxyli* is significantly different from its sister species, *Do. camelliae* by 4/550 bp (0.72%) in ITS and 14/242 bp (5.8%) in *tef1*. In the phylogenetic analysis, these two species formed two distinct clades in *Dothiorella* (Fig. 4).

***Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, Stud. Mycol. 55: 248. (2006).**

MycoBank No: 500879

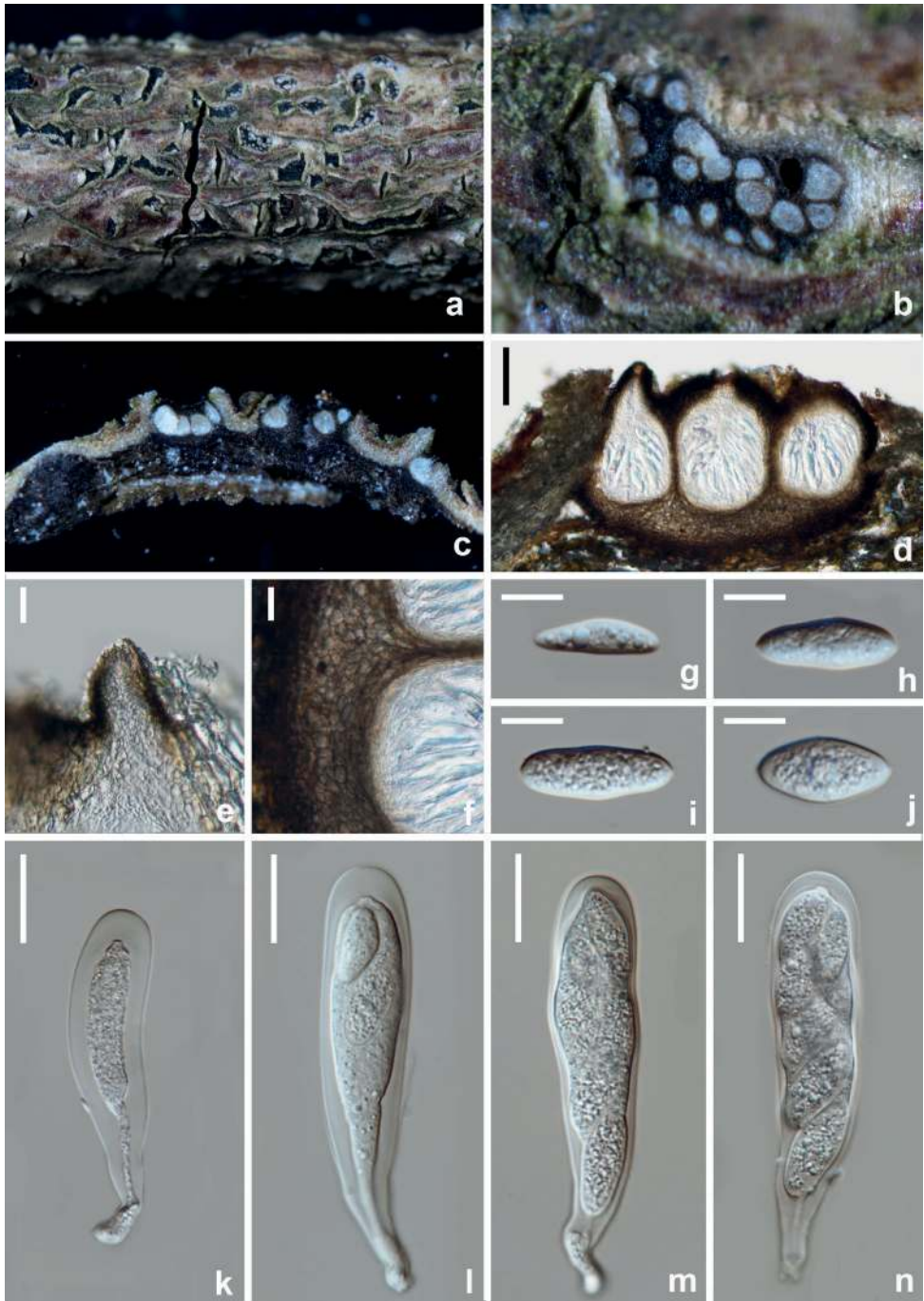
Fig. 15

*Fusicoccum parvum* Pennycook & Samuels, Mycotaxon 24: 455. 1985. Basionym.  
= *Botryosphaeria parva* Pennycook & Samuels, Mycotaxon 24: 455. 1985.

**Description.** *Saprobic* on decaying branches of *Idesia polycarpa*. **Sexual morph:** **Ascomata** 284–321 × 129–223 µm ( $\bar{x}$  = 302.5 × 176 µm, n = 10), pseudothecial, forming a botryose aggregation of up to 30, solitary or gregarious, stromatic, immersed, partially erumpent when mature, dark brown to black, more or less circular, multi-loculate, individual locules 143.5–161 µm diam, thick-walled. **Peridium** 59–78 µm diam., composed of several layers of thick-walled, pale brown cells of *textura angularis*. **Ostiole** 43.5–58 µm wide, circular, central, papillate. **Asci** 95–99 × 20–21.5 µm ( $\bar{x}$  = 97 × 20.5 µm, n = 30), (6–)8-spored, bitunicate, fissitunicate, cylindrical to clavate, apex rounded with an ocular chamber, sometimes short pedicellate. **Ascospores** 18.5–23 × 7–10.5 µm ( $\bar{x}$  = 20.5 × 9 µm, n = 30), L/W ratio = 3, fusoid to ovoid, with tapered ends and appearing spindle-shape, hyaline, aseptate, externally smooth, internally finely verruculose, biseriate in ascus. **Asexual morph:** Not observed.

**Culture characteristics.** Ascospores germinate on PDA within 12 h. Colonies growing on PDA, reaching a diam., of 7 cm after five days at 25 °C, effuse, velvety, with entire to slightly undulate edge. Surface initially white and later turning dark olivaceous from the surrounding of the colony and dark gray in reverse.

**Materials examined.** CHINA, Sichuan Province, Leshan City, Jingyan County, 29°30'27"N, 103°57'14"E, elevation 682 m, on dead branches of *Idesia polycarpa*, 23<sup>th</sup>



**Figure 15.** *Neofusicoccum parvum* (HUEST 22.0097, new host record) **a, b** appearance of ascomata on natural substrate **c, d** vertical section of ascomata **e** ostiole **f** section of peridium **g–j** ascospores **k, l** immature asci **m, n** mature asci. Scale bars: 100  $\mu\text{m}$  (**d**); 25  $\mu\text{m}$  (**e, f**); 10  $\mu\text{m}$  (**g–j**); 20  $\mu\text{m}$  (**k–n**).

July 2021, W.L. Li, STZ 327 (HUEST 22.0095), living culture UESTCC 22.0094; *ibid.*, STZ 359 (HUEST 22.0094), living culture UESTCC 22.0093; *ibid.*, Leshan City, Shizhong District, 29°42'13"N, 103°52'25"E, elevation 356 m, on dead branches of *Paeonia suffruticosa*, 23<sup>rd</sup> July 2021, W.L. Li, YMD 366 (HUEST 22.0096), living culture UESTCC 22.0095; *ibid.*, Guangyuan City, Qingchuan County, 32°40'38"N, 105°28'57"E, elevation 638 m, on dead branches of *Vernicia fordii*, 20<sup>th</sup> April 2021, W.L. Li, YT 175 (HUEST 22.0097), living culture UESTCC 22.0096.

**Notes.** The morphology of our collections obtained from decaying woody oil plants are similar to the original description of *Neofusicoccum parvum* (Crous et al. 2006). In the multi-gene phylogenetic analysis, these four isolates clustered together (ML/BI 75%/0.99) with the ex-type of *N. parvum*. *Neofusicoccum parvum* has a wide range of hosts and has a worldwide distribution (Phillips et al. 2013). This is the first report of *N. parvum* on *Idesia polycarpa*.

***Sardiniella guizhouensis* Y.Y. Chen & Jian K. Liu. Phytotaxa 508 (2): 190. (2021).**  
MycoBank No: 558352

Fig. 16

**Description.** *Saprobic* on decaying branches of *Pistacia chinensis*. **Sexual morph:** Not observed. **Asexual morph:** **Conidiomata** 223–232 × 150–176 µm ( $\bar{x}$  = 227.5 × 163 µm, n = 10), dark brown to black, globose, submerged in the substrate, partially erumpent at maturity, ostiolate. **Ostiole** 28.5–45 µm diam., circular, central. **Peridium** 21–30 µm thick, composed of dark brown thick-walled cells of *textura angularis*, becoming thin-walled and hyaline towards the inner region. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** 6–9.5 × 3.5–5 µm ( $\bar{x}$  = 7.5 × 4 µm, n = 20), hyaline, short obpyriform to subcylindrical, holoblastic, indeterminate. **Conidia** 20.5–24 × 11.5–14 µm ( $\bar{x}$  = 22 × 13 µm, n = 30), L/W ratio = 1.6, ellipsoid to ovoid with both ends rounded, hyaline, aseptate, externally smooth, internally finely verruculose.

**Culture characteristics.** Conidia germinate on PDA within 12 h. Colonies growing on PDA, reaching a diameter of 7 cm after five days at 25 °C, effuse, velvety, with entire to slightly undulate edge. Surface initially white and later turning dark olivaceous from the surrounding of the colony and dark gray in reverse.

**Material examined.** CHINA, Sichuan Province, Chengdu City, Pidu District, 29°16'50.70"N, 102°37'47.53"E, elevation 442 m, on dead branches of *Pistacia chinensis*, 19<sup>th</sup> March 2021, W.L. Li, 047 (HUEST 22.0101), living culture UESTCC 22.0100; *ibid.*, 070 (HUEST 22.0102), living culture UESTCC 22.0101; *ibid.*, 071 (HUEST 22.0100), living culture UESTCC 22.0099; *ibid.*, 150 (HUEST 22.0098), living culture UESTCC 22.0097; *ibid.*, 151 (HUEST 22.0099), living culture UESTCC 22.0098; *ibid.*, A39 (HUEST 22.0103), living culture UESTCC 22.0102; *ibid.*, A40 (HUEST 22.0104), living culture UESTCC 22.0103. Additional



**Figure 16.** *Sardiniella guizhouensis* (HUEST 22.0100, new host record) **a–c** appearance of conidiomata on natural substrate **d** vertical section of conidioma **e** section of peridium **f** ostiole **g–j** conidiogenous cells and developing conidia **k–n** conidia. Scale bars: 40  $\mu\text{m}$  (**d**); 20  $\mu\text{m}$  (**e**, **f**); 10  $\mu\text{m}$  (**g–n**).

sequences: LSU: OQ164842 (UESTCC 22.0100), OQ164843 (UESTCC 22.0101), OQ164844 (UESTCC 22.0099), OQ164845 (UESTCC 22.0097), OQ164846 (UESTCC 22.0098), OQ164847 (UESTCC 22.0102).

**Notes.** Seven isolates of our collection are morphologically similar to the original description of *Sardiniella guizhouensis* (Chen et al. 2021). The multi-gene phylogenetic

analysis showed that the newly obtained isolates clustered together with ex-type of *Sa. guizhouensis* (CGMCC 3.19222) and this is the first report of *Sa. guizhouensis* from *Pistacia chinensis*.

***Sphaeropsis citrigena* (A.J.L. Phillips, P.R. Johnst. & Pennycook) A.J.L. Phillips & A. Alves. Stud. Mycol. 76, 157. (2013).**

MycoBank No: 805463

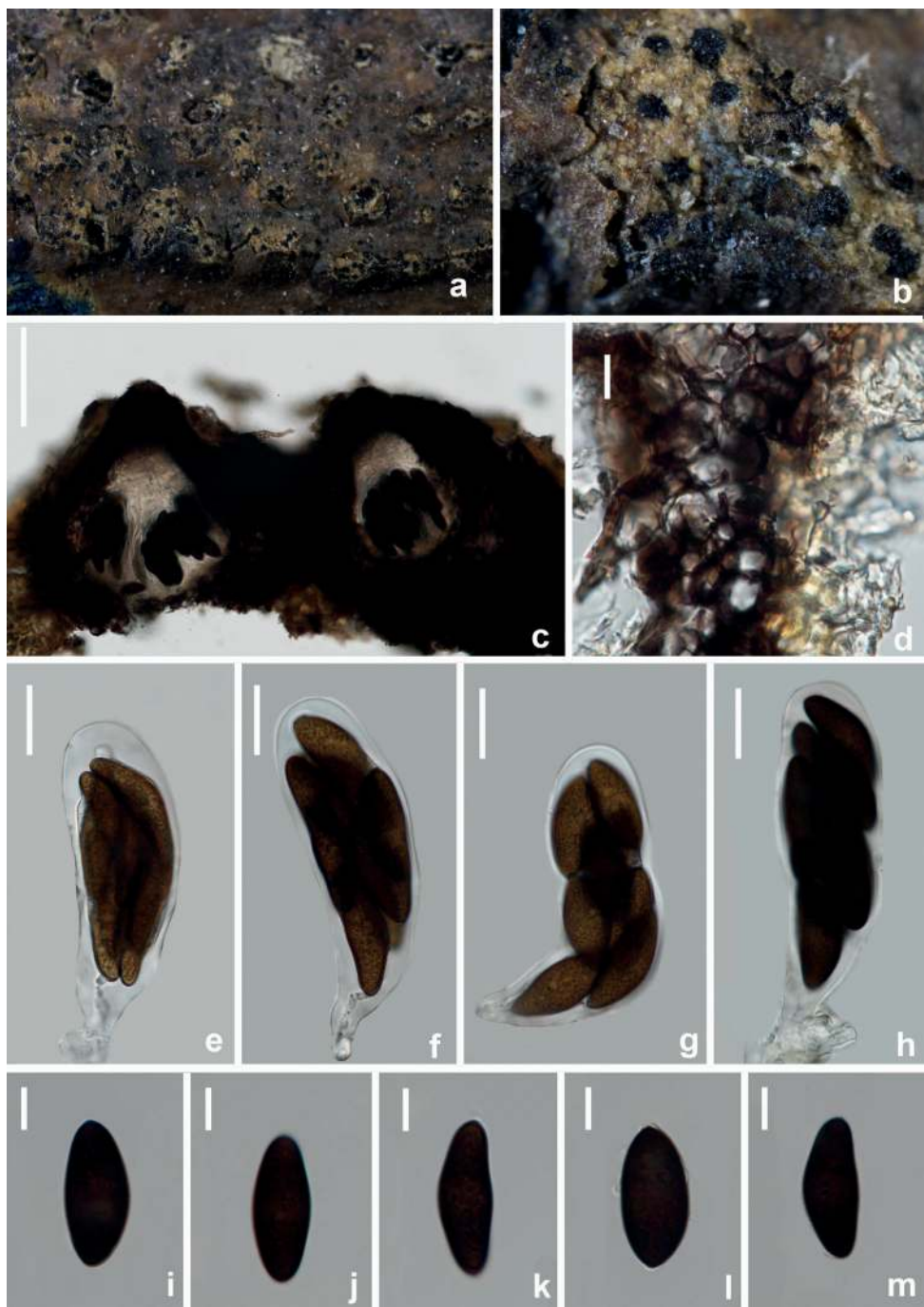
Fig. 17

**Description.** *Saprobic* on decaying branches of *Camellia oleifera*. **Sexual morph:** **Ascomata** 219–252 × 216–241 µm ( $\bar{x}$  = 235.5 × 228.5 µm, n = 10), brown to black, solitary or aggregated, immersed, becoming erumpent, ostiolate. **Ostiole** 71–92 µm, central, relatively broad. **Peridium** 37.5–45 µm diam., composed of several layers of dark brown cells of *textura angularis*. **Pseudoparaphyses** 1.5–2 µm wide, hyaline, smooth, septate. **Asci** 93.5–107 × 28.5–33 µm ( $\bar{x}$  = 100 × 30.5 µm, n = 30), bitunicate, 8-spored, stipitate, thick-walled, with well-developed apical chamber. **Ascospores** 29–35 × 13–15 µm ( $\bar{x}$  = 32 × 14 µm, n = 30), L/W ratio = 2.3, yellowish brown to dark brown, ellipsoid to ovoid with both ends rounded, with an apiculus at either end, aseptate, externally smooth, internally finely verruculose, widest in middle to upper third. **Asexual morph:** Not observed.

**Culture characteristics.** Ascospores germinate on PDA within 12 h. Colonies growing on PDA, reaching a diam. of 7 cm after five days at 25 °C, effuse, velvety, with entire to slightly undulate edge. Surface initially white and later turning dark olivaceous from the surrounding of the colony and dark gray in reverse.

**Materials examined.** CHINA, Sichuan Province, Chengdu City, Pidu District, 31°54'10"N, 104°55'57"E, 656 m, on dead branches of *Camellia oleifera*, 10<sup>th</sup> June 2021, W.L Li, 285 (HUEST 22.0107), living culture UESTCC 22.0106; *ibid.*, on dead branches of *Acer truncatum*, 30°19'57"N, 103°59'47"E, elevation 442 m, 19<sup>th</sup> March 2021, W.L Li, A33 (HUEST 22.0106), living culture UESTCC 22.0105. Additional sequence: LSU: OQ164848 (UESTCC 22.0105).

**Notes.** The phylogenetic tree shows that two isolates of *Sphaeropsis* from our collection clustered together with the ex-type strain of *Sp. citrigena* (ICMP 16812) with high bootstrap support (ML/BI 100%/1). *Sphaeropsis citrigena* was first described as *Phaeobotryosphaeria citrigena* by Phillips et al. (2008), later transferred to *Sphaeropsis* based on morphological and phylogenetic analyses (Phillips et al. 2013). The new collection (UESTCC 22.0105) isolated from *Camellia oleifera* resembles *Sp. citrigena* isolated from *Citrus sinensis* (Phillips et al. 2013) in the shape of asci and ascospores, though their asci are somewhat smaller than those of *Sp. citrigena* (93.5–107 × 28.5–33 µm vs. 180–230 × 35–43 µm). In addition, there are no base pair differences in ITS and *tef1* sequences of these two strains. We, thus, identify the new collection as *Sp. citrigena* and this is the first record of *Sp. citrigena* from *Camellia oleifera*.



**Figure 17.** *Sphaeropsis citrigena* (HUEST 22.0107, new host record) **a, b** appearance of ascomata on natural substrate **c** vertical section of ascomata **d** section of peridium **e–h** mature asci **i–m** dark brown ascospores. Scale bars: 100 µm (**c**); 20 µm (**d–h**); 10 µm (**i–m**).

***Sphaeropsis guizhouensis* Y.Y. Chen, A. J. Dissanayake & Jian K. Liu., J. Fungi 7, 893. (2021).**

MycoBank No: 558475

Fig. 18

**Description.** *Saprobic* on decayed branched of *Camellia oleifera*. **Sexual morph:** *Ascostromata* 166–198 × 146.5–175 µm ( $\bar{x}$  = 182 × 160.5 µm, n = 20), initially immersed under host epidermis, becoming semi-immersed to erumpent, solitary or gregarious, uniloculate, black, globose to subglobose, membranous, ostiolate. *Ostiole* 75–80 µm wide, central, papillate, pale brown, relatively broad, periphysate. *Peridium* 23–27 µm wide, comprising 3–5 layers of relatively thick-walled, dark brown to black-walled cells arranged in a *textura angularis*. *Pseudoparaphyses* 2–2.5 µm diam., hyphae-like, numerous, embedded in a gelatinous matrix. *Asci* 87.5–135 × 28.5–35 µm ( $\bar{x}$  = 111 × 32 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, sometimes short pedicellate, mostly long pedicellate, apex rounded with an ocular chamber. *Ascospores* 28.5–33 × 13–15 µm ( $\bar{x}$  = 30.5 × 14 µm, n = 20), overlapping uniseriate to biseriate, ellipsoidal to obovoid, pale brown to dark brown, septate, slightly wide at the center, minutely guttulate, smooth-walled. **Asexual morph:** Not observed.

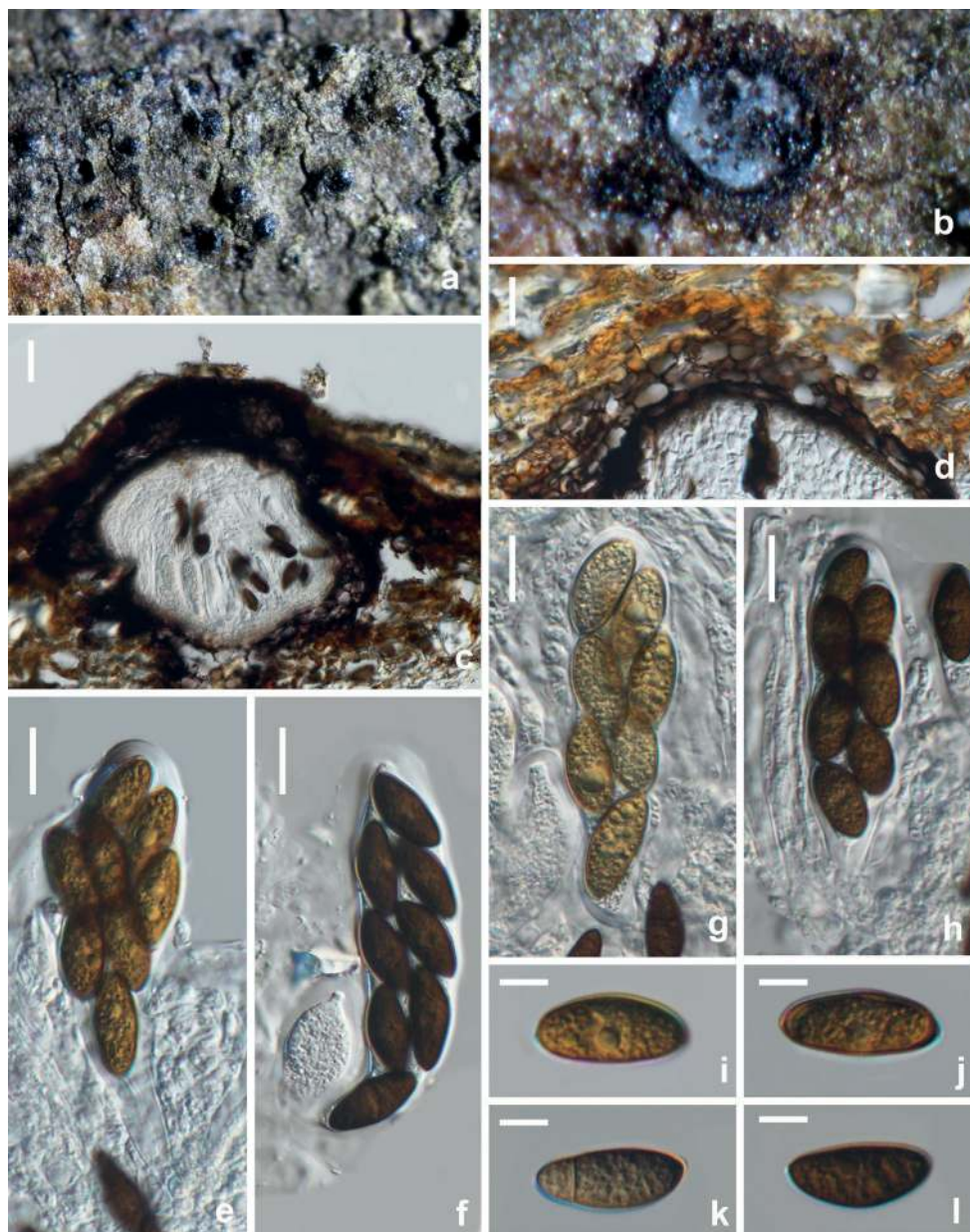
**Culture characteristics.** Ascospores germinate on PDA within 12 h. Colonies growing on PDA, reaching a diam. of 7 cm after five days at 25 °C, effuse, velvety, with entire to slightly undulate edge. Surface initially white and later turning dark olivaceous from the surrounding of the colony and dark gray in reverse.

**Material examined.** CHINA, Sichuan Province, Chengdu City, Pidu District, on dead branches of *Pistacia chinensis*, 30°19'57"N, 103°59'47"E, elevation 442 m, 24<sup>th</sup> March 2021, W.L Li, 290 (HUEST 22.0105), living culture UESTCC 22.0104.

**Notes.** *Sphaeropsis guizhouensis* was introduced by Dissanayake et al. (2021) and isolated from an unknown host. One isolate obtained in the present study clustered with the ex-type isolate of *Sp. guizhouensis* (CGMCC 3.20352) in the phylogenetic analyses of combined ITS and *tef1* sequence data with high bootstrap support. A comparison of ITS and *tef1* shows that there are no base pair differences between the isolates of UESTCC 22.0104 and CGMCC 3.20352. The new collection is morphologically similar to *Sp. guizhouensis*, with immersed to erumpent, black ascostromata and biseriate, aseptate, ellipsoid to obovoid, thick-walled conidia. In addition, ascospores become brown and septate when aged. Considering similar morphology and strong molecular evidence, we identify UESTCC 22.0104 as *Sp. guizhouensis* and this is the first record of *Sp. guizhouensis* on *Camellia oleifera*.

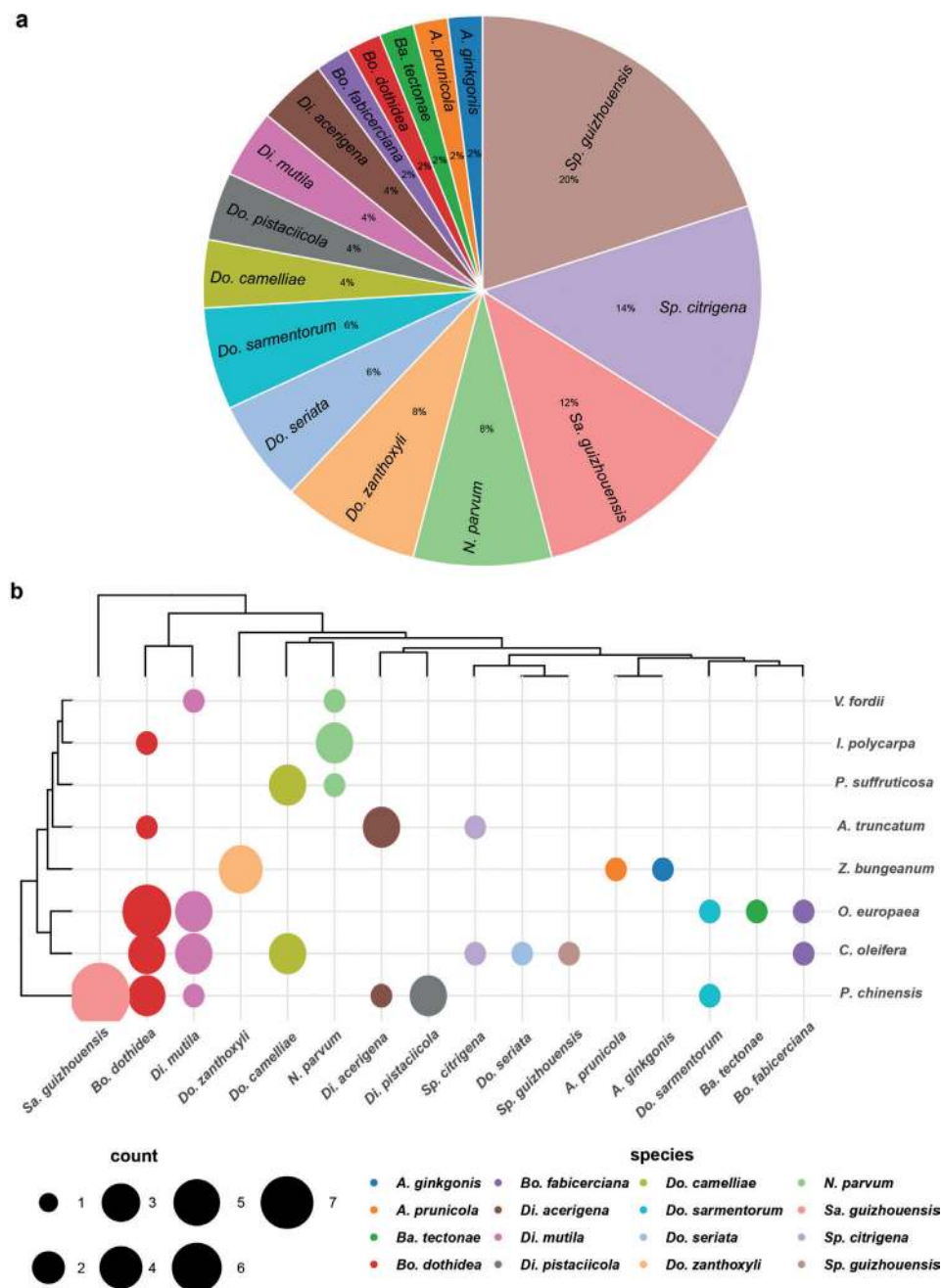
## Diversity of Botryosphaeriales fungi collected in this study

Based on the phylogenetic and morphological analyses, 50 Botryosphaeriales isolates collected from the five regions (Chengdu, Guangyuan, Leshan, Mianyang and Yaan City) in Sichuan Province were identified as 16 species. Of these, *Botryosphaeria dothidea* was the most prevalent species (20%), followed by *Sphaeropsis guizhouensis*



**Figure 18.** *Sphaeropsis guizhouensis* (HUEST 22.0105, new host record) **a, b** appearance of ascomata on natural substrate **c** vertical section of ascoma **d** section of peridium **e–h** mature asci **i–l** brown ascospores. Scale bars: 20  $\mu$ m (**c–h**); 5  $\mu$ m (**i–l**).

(14%) and *Diplodia mutila* (12%) (Fig. 19a). *Aplosporella ginkgonis*, *Barriopsis tectonae* and *Sphaeropsis guizhouensis* were identified only once. There are 14 isolates (28%) isolated from *Pistacia chinensis*, including *Di. acerigena*, *Di. mutila*, *Di. pistaciicola*,



**Figure 19.** Botryosphaerales species composition **a** the proportion of each species to the total number of isolates **b** the number of Botryosphaerales fungi on each host and host distribution of species.

*Dothiorella sarmentorum* and *Sardiniella guizhouensis*. Ten isolates were from *Camellia oleifera* (20%), including *Bo. dothidea*, *Bo. fabicerciana*, *Di. mutila*, *Do. camelliae*, *Sp. citrigena* and *Sp. guizhouensis*. Nine isolates were from *Olea europaea* (18%),

including *Ba. tectonae*, *Bo. dothidea*, *Bo. fabicerciana*, *Di. mutila* and *Do. sarmentorum*. Relatively few strains were found on *Idesia polycarpa*, *Paeonia suffruticosa* and *Vernicia fordii*, as each host presents two species, respectively. As of final conclusion, *Bo. dothidea* were isolated from five hosts, *Di. mutila* were isolated from four hosts, *N. parvum* were isolated from three hosts, *Bo. fabicerciana*, *Di. acericola*, *Do. camelliae*, *Do. sarmentorum* and *Sp. citrigena* were isolated from two hosts, but several fungal isolates were only isolated from one host species, such as *A. prunicola*, *Sa. guizhouensis* and *Sp. guizhouensis* (Fig. 19b).

## Discussion

In this study, 48 Botryosphaeriaceae isolates and two Aplosporellaceae isolates were obtained from woody oil plants in Sichuan Province, China, and they were identified as 16 species based on morphological characters and multi-gene phylogenetic analyses. These species included *Aplosporella prunicola*, *A. ginkgonis*, *Barriopsis tectonae*, *Botryosphaeria dothidea*, *Bo. fabicerciana*, *Diplodia acerigena*, *Di. mutila*, *Di. pistaciicola*, *Di. seriata*, *Dothiorella camelliae*, *Do. sarmentorum*, *Do. zanthoxyli*, *Neofusicoccum parvum*, *Sardiniella guizhouensis*, *Sphaeropsis citrigena* and *Sp. guizhouensis*. Of these, *Di. acerigena*, *Di. pistaciicola*, *Do. camelliae* and *Do. zanthoxyli* are introduced as novel species. Descriptions, illustrations and notes were provided for 13 species, and only sequences data were provided for the remaining three species viz. *Barriopsis tectonae*, *Botryosphaeria dothidea* and *Bo. fabicerciana* due to low specimen quality.

According to previous studies, *Barriopsis tectonae*, *Sardiniella guizhouensis*, *Sphaeropsis citrigena* and *Sp. guizhouensis* have limited geographical distribution. So far, *Barriopsis tectonae* has been reported from China, Thailand and South Africa (Doilom et al. 2014; Dissanayake et al. 2021). *Sardiniella guizhouensis* and *Sphaeropsis guizhouensis* were only found in China while *Sp. citrigena* was isolated from China, Colombia and New Zealand. It's worth noting that most of the species obtained from this study were also reported previously from Guizhou province (Dissanayake et al. 2021). Earlier studies have shown that the distribution of Botryosphaeriaceae species is influenced by the climate condition (Úrbez-Torres et al. 2006; Pitt et al. 2010; Li et al. 2020; Vivas et al. 2021). Thus, we speculate that the adjacent geographical location and similar climatic conditions may be important reasons for the similarity of fungal species isolated from the Sichuan and Guizhou provinces.

The remaining Botryosphaeriaceae species identified in this study are all well-known and reported from various geographic regions. *Botryosphaeria dothidea*, *Di. seriata* and *Ne. parvum* are recognized to be globally distributed while *Di. mutila* and *Do. sarmentorum* are founded only in the temperate and Mediterranean areas. In addition, these species have a broad host range. Batista et al. (2021) reported *Neofusicoccum parvum* from 223 hosts, *B. dothidea* from 403 hosts and *Di. seriata* from 121 hosts. *Diplodia mutila* and *Di. seriata* have previously been reported on *Olea europaea* in Uruguay (Hernández-Rodríguez et al. 2022). *Botryosphaeria dothidea* was recently isolated from diseased *Camellia oleifera* in China (Hao et al. 2022). In this study, *Bo. dothidea*,

*Di. mutila* and *Ne. parvum* occurred on most of the woody oil plants species we examined. However, some common genera e. g. *Lasiodiplodia*, *Neodeightonia* and *Phaeobotryon* have never been collected from this group of hosts (Fig. 19). The absence of these genera from there is likely a sampling effect.

*Aplosporella* (Aplosporellaceae) was introduced by Spegazzini (1880) with *A. chlorostroma* as the genetic type. In a previous study, *Aplosporella* represents anamorph lineage within the Botryosphaeriaceae. Slippers et al. (2013) later proposed the family Aplosporellaceae to accommodate *Aplosporella* and *Bagnisiella*. *Aplosporella* species are infrequently isolated in China. *Aplosporella ginkgonis*, isolated from Gansu Province, was first described by Du et al. (2017) while *Aplosporella macropycnidia* was reported in Yunnan Province. Subsequently, Jiang et al. (2021) isolated a new collection of *A. prunicola*. However, other species have not been recorded in China. Our study revealed new host records of *A. ginkgonis* and *A. prunicola*. Though the phylogenetic analyses indicated that *A. yalgorensis* and *A. prunicola* have a low genetic divergence (Taylor et al. 2008, in this study), *A. yalgorensis* is still considered as a different species as it differs from other *Aplosporella* species (including *A. prunicola*) by its pitted conidial walls.

Though there are more than 1,000 *Diplodia* epithets listed in Index fungorum (www. Index Fungorum. Accessed in November 2022), presently only 30 species are accepted in this genus based on phylogenetic analyses (Slippers et al. 2017; Wu et al. 2021). Holomorphic species in *Diplodia* are *Di. tsugae*, *Di. seriata*, *Di. mutila* and *Di. sapinea*. This study revealed two previously known *Diplodia* species, *Di. mutila* and *Di. seriata*, and two new species, *Di. acerigena* and *Di. pistaciicola*. Among them, *Di. acerigena* is a holomorphic species, as its sexual stage was observed on the dead branches of *Acer truncatum*, and the asexual stage produced on culture (PDA). However, the sexual morph of *Di. mutila* and *Di. pistaciicola*, as well as the asexual morph of *Di. seriata* have not been observed on woody oil plants.

*Dothiorella* was established by Saccardo with *Do. pyrenophora* as the type species (Saccardo 1880). Recently, *Dothiorella* encountered a series of revisions as many species in this genus have been reduced to synonymy, such as *Do. americana*, *Do. eriobotryae* and *Do. iberica* (Dissanayake et al. 2021; Zhang et al. 2021). So far, 31 species are valid in *Dothiorella*. Most of the species were reported as the asexual morph of *Dothiorella* and the sexual stage is rarely founded on nature (Dissanayake et al. 2016). Phillips et al. (2013) initiated a link of asexual-sexual morph for *Do. sarmentorum*, *Do. iberica* and *Do. vidmaderae*. However, the latter two species were synonymized under *Do. sarmentorum* (Zhang et al. 2021). In this study, two new species *Do. camelliae* and *Do. zanthoxyli* are introduced based on their sexual morphs as well as strong molecular evidences. Besides, new collections of *Do. sarmentorum* is reported on *Pistacia chinensis* for the first time.

Multiple molecular systematic studies, mainly of pathogenic fungi of woody plants (Phillips et al. 2013; Slippers et al. 2013; Dissanayake et al. 2021; Zhang et al. 2021), have generated a robust phylogeny for Botryosphaeriaceae. However, the classification and identification of some species in this family remains a major challenge, due to the reasons 1) With the increase of the number of Botryosphaeriaceae species, morphological feature of inter-genera and inter-species is vague, 2) Some species occurred as asexual morph on nature and it is difficult to establish the link of asexual and sexual

morph, 3) In general, Botryosphaeriaceae species do not show an obvious host specialization, while some populations displayed a certain degree of host association. Thus, the traditional host-based classification system made taxonomic position confusion of some species. Therefore, collection of more fresh specimens is very important for better understanding the life cycle of Botryosphaeriaceae species, their host range (e. g. native plants) and potential pathogenicity.

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## Supplementary material I

### **Taxa and GenBank accession numbers of sequences used in this study**

Authors: Wen-Li Li, Rui-Ru Liang, Asha Dissanayake, Jian-Kui Liu

Data type: table (excel document)

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